















October 1, 1985 to September 30, 1986

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT  
INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR  
OCTOBER 1, 1985 TO SEPTEMBER 30, 1986

The Intramural Research Program is broadly concerned with the biological and neurobiological, medical and behavioral aspects of normal and abnormal human development. In addition to four major clinical research and training programs in the areas of genetics and endocrinology, a diversity of developmental models are under study in twelve fundamental research Laboratories, drawing upon observations in bacteria, Drosophila, yeasts, viruses, molluscs, frogs, rodents, and subhuman primates. Disciplines employed in these studies include biochemistry, virology, molecular biology, immunology, pharmacology, genetics, cell and neuronal biology, biophysics, mathematical and theoretical biology, reproductive physiology, and comparative ethology.

During the past year, we have continued to strengthen a number of research programs that we believe to have a particular promise and timeliness. In these areas, new resources have been added so that the investigators will be optimally poised to exploit their recent findings. These programs address questions in cell and molecular biology, wherein we anticipate significant results with broad implications for biomedical research. One topic of great interest is pursued by the Cell Biology and Metabolism Branch, which focuses on the developmental and regulatory aspects of various cellular organelles and receptors. Two groups of investigators in this Branch are studying structure-function relationships as well as molecular regulation of interleukin-2 (T cell growth factor) receptors. A similar approach is being taken to studies on the T cell antigen receptor. Both types of receptors influence the proliferation and activity of immunocytes, and anomalies in their structure or regulation may underlie many immunological disorders.

The Laboratory of Molecular Genetics is expanding its interest in homeotic genes, which specify the spatial and temporal parameters of organ and limb morphology during development of the fruit fly, Drosophila melanogaster. Recently, it has become apparent that these very important genes, which play a critical role in normal embryogenesis, have been highly conserved through evolution, and homologous genes appear to be present in humans. The LMG is also continuing to expand its work in yeast genetics. Yeast is a eukaryotic organism which offers a level of genetic and molecular analysis that is difficult to achieve in higher eukaryotes. Moreover, many essential genetic mechanisms and biochemical pathways manifested in the human are demonstrable in this organism. Much emphasis has also been given to work on the transgenic mouse in the LMG. This work has flourished during the past year, and many new transgenic mouse strains have been established, manifesting the addition or deletion of heritable characteristics. The transgenic mouse model presents extraordinary opportunities for the study of spatial and temporal gene regulation, particularly during mammalian development, but the work also serves as a prelude to gene therapy in humans.

An area that has received much recent attention, in both the Laboratory of Developmental Neurobiology and the Laboratory of Neurochemistry and Neuroimmunology, relates to the cell biology of brain development, especially the



hormonal and electrical phenomena that endow the mammalian brain with its rich but still not well-appreciated structural and functional plasticity. Tools have also become available to apply classical molecular biological techniques to the study of gene regulation in the nervous system, and we plan to pursue this area vigorously.

In clinical research, the Human Genetics Branch has been intensifying its efforts in the diagnosis and treatment of human genetic diseases, especially osteogenesis imperfecta and various inborn errors of metabolism such as cystinosis. Here too, the availability of powerful new molecular and cell biologic tools is permitting our investigators to illuminate the underlying mechanisms of these diseases and to devise new therapies.

The Laboratory of Developmental and Molecular Immunity has expanded both its applied and basic studies. Much effort has gone into the development of bacterial vaccines, the design of which is predicated upon an understanding of the development of the immune system in infants and children. In particular, the group responsible for these efforts has focused on new vaccines directed against pertussis, H. influenzae, Salmonella typhi, and meningococcus--all agents with great consequences for the public health. At the more basic level, the LDMI has moved heavily into studies on the molecular regulation of genes which influence important immunological phenomena such as the expression of class I mixed histocompatibility (MHC) antigens, and the interferon system.

A number of new areas in endocrinology have received particular attention during the past year, again ranging from the applied to the basic. At the more applied level, the Laboratory of Theoretical and Physical Biology has expanded its efforts in the development of algorithms and computer programs that guide the therapeutic management of chronic disorders such as diabetes. The programs have the potential to greatly refine treatment regimens, and also encourage the patient with access to a computer to take a more active role in his or her own treatment. The national data base being accrued through the use of these programs is clearly an invaluable clinical research tool. At the more basic level, much new effort in the Endocrinology and Reproduction Research Branch, as well as the Developmental Endocrinology Branch, has been devoted to cell biologic and molecular studies which exploit the growing awareness that hormones and releasing factors, formally thought to have roles only within the endocrine axis, in fact have far broader roles, particularly with respect to the integration of global brain responses such as occur in stress, hypertension, and depression. In clinical research, the Developmental Endocrinology Branch is assessing the new generation of therapeutic hormones, e.g., growth hormone, produced by recombinant DNA technology.

The Laboratory of Comparative Ethology, established late in 1983, continues to undergo a major building program. An extensive outdoor facility for free-ranging primates, employed in observational studies on the genetics of behavior, is now in place, and construction of a three-floor indoor facility, including breeding quarters and a newborn nursery, has been recently completed. These facilities are located at the NIH's Animal Center in Poolesville, Maryland.

The Laboratory of Developmental Pharmacology, which has (for almost two decades) focused on the cytochrome P450-associated enzyme system (highly conserved from prokaryotes to man), is fast approaching the development of molecular-



biology based assays for determining the individual genetic polymorphisms which influence the function of this system. The P450 enzymes are responsible for the metabolism and detoxification of endogenous ligands as well as countless environmental chemicals which can cause birth defects, cancers, and idiosyncratic drug responses. Intensive effort is being put into the cloning and sequencing of the many genes involved in the P450-system, so as to develop useful probes for individual risk assessment, e.g., the risk of lung cancer faced by a given cigarette smoker.

Finally, the laboratories of the Scientific Director and Deputy Scientific Director have expanded their studies on growth factors, especially nerve growth factor (NGF) and transforming growth factors (TGF's), as it has become evident that these factors play critical but complex roles in normal development as well as such diseases as cancer and chronic neurological disorders. Much effort is also being directed to studies on the mechanism of mammalian mutagenesis, taking advantage of new recombinant DNA tools such as "shuttle vectors."

The Institute continues to acquire additional laboratory space. Construction for a three-floor addition to Building 6 is almost completed, and will provide twenty-five new laboratory modules, as well as an extensive animal facility. This new space will accommodate the Laboratory of Developmental Pharmacology, one section of the Laboratory of Developmental and Molecular Immunity, and the DNA/protein Sequencing and Synthesis Unit of the Endocrinology and Reproduction Research Branch. When these moves are complete, we will be able to move the Laboratory of Theoretical and Physical Biology into larger, centralized quarters, and as well, we shall increase the amount of laboratory space assigned to a number of other Laboratories and Branches located in the Clinical Center, including the Human Genetics Branch, the Developmental Endocrinology Branch, and the Endocrinology and Reproduction Research Branch. Within the neuroscience complex (Buildings 36 and 37) we have recently added twenty additional modules for neurobiology research. The space is now being renovated and should be ready for occupancy in 1987. Plans are underway also for a large additional animal facility in the vicinity of Building 6 (Bldg. 6 "B").

In major personnel changes during the past year, Dr. William Gahl was granted tenure in the Human Genetics Branch and Dr. Fernando Cassorla in the Developmental Endocrinology Branch. Dr. Richard Knazek transferred to the Developmental Endocrinology Branch from the NCI, and Dr. Robert Klein left the Laboratory of Comparative Ethology to join the Division of Computer Research Technology.

Our clinical and laboratory research fellowships for physicians in adult, pediatric, and reproductive endocrinology, as well as the fellowship in medical genetics, continue to thrive, and in the past year, we have continued to develop new sources of support for post-doctoral (Ph.D. and M.D.) fellows. These funds include post-doctoral stipends paid by the Institute but which do not encumber regular government positions. We anticipate that these new mechanisms will greatly enhance our ability to offer post-doctoral training. The new mechanisms include an Intramural National Research Scholarship Award (NRSA) program, a contractual program for the support of intramural training in biotechnology administered by the National Research Council of the National Science Foundation, and a new fellowship program which will begin October 1, 1986 that is intended for American post-doctoral candidates (Ph.D.s or M.D.s). The latter mechanism is the domestic equivalent of our long-standing Visiting

Fellow Program for post-doctoral candidates from abroad. The new program (Intramural Research Training Award, or "IRTA") will have no ceiling other than available funds. We have also been successful in identifying new donors of stipends, including endowments by private industry and foundations. Contractual funds administered by the NIH's Fogarty International Center have been employed for the support of sabbatical visits by senior scientists from abroad. Moreover, a number of foreign post-doctoral fellows have been awarded stipends for training in our laboratories under the terms of formal bilateral agreements generated between the NIH and several countries in Europe, Asia, and the Middle East. Finally, a number of medical students supported by the new Howard Hughes Foundation program at the NIH are working in our laboratories during an elective year, and our Summer Student Program continues to be very successful, with more than fifty undergraduate, graduate, and medical students working in our laboratories this year, despite the fact that most were here on a volunteer basis.

Peer review of intramural research, conducted by the Institute's Board of Scientific Counselors and ad hoc experts, continues to receive great emphasis, with rigorous site visits to each Lab at four year intervals. During the past year, visits were made to the Human Genetics Branch, the Laboratory of Neurochemistry and Neuroimmunology, and the Laboratory of Developmental and Molecular Immunity, with detailed critiques prepared as a consequence of these visits. The membership of the Board of Scientific Counselors reflects the increasing diversity of research interests within this Intramural Program. The current Board membership includes:

Joseph G. Gall, Ph.D., Senior Member, Department of Embryology, Carnegie Institution of Washington (Chairman)

Stanley Cohen, Ph.D., Professor, Department of Biochemistry, Vanderbilt University

John C. Marshall, M.D., Ph.D., Professor of Medicine, University of Michigan

Lewis P. Lipsitt, Ph.D., Professor of Psychology, Brown University

Allen H. Neims, M.D., Ph.D., Professor and Chairman, Department of Pharmacology and Therapeutics, University of Florida

Story C. Landis, Ph.D., Professor, Department of Neurobiology, Case Western Reserve University

Merry R. Sherman, Ph.D., Professor of Biochemistry, Rutgers University

Harold Amos, Ph.D., Professor of Bacteriology and Immunology, Harvard Medical School

John Phillips, Jr., M.D., Professor of Human Genetics, Vanderbilt University School of Medicine

Other developments in the past year include the continued strengthening of all aspects of the management of animals employed in our research. Dr. John Donovan, the Institute's full-time veterinarian, left during the year to become Chief Veterinarian for the National Cancer Institute, but we have been fortunate to recruit Dr. William Stokes from the U.S. Army Medical Research Command to fill this position. We have continued to develop new computer-based administrative procedures in the Office of the Scientific Director so as to maximize the efficiency with which our resources are deployed. These new administrative approaches are ensuring the maximum yield with respect to scientific productivity while the current climate of constrained resources persists.



Seminars by the twelve Laboratories and Branches in this Program were numerous and well attended throughout the year, such that this Institute organized a relatively large fraction of the NIH's overall offering of intramural seminars and workshops. During the past year also, three major international conferences were hosted by Laboratories of the Intramural Research Program on the Bethesda campus or in the surrounding area, including;

The Molecular Genetics and Development in Frogs, Flies, and Mice (Laboratory of Molecular Genetics)

Mechanisms of Physical and Emotional Stress (Developmental Endocrinology Branch)

Advances in Research on Human Growth (Developmental Endocrinology Branch)

Another meeting, on developmental biology, was co-organized by this Institute and the Centre National de la Recherche Scientifique, and held in Paris during October, with much intramural participation. During the coming year, in honor of the NIH's centennial, we plan major conferences on the Cytochrome P450 System, Neuronal Plasticity, the Molecular and Developmental Regulation of the Immune System, and the Biochemistry of Receptor Activation.

During the year, we were especially honored by the award to Dr. Daniel Nebert (Chief, Laboratory of Developmental Pharmacology) of the Bernard Brodie Award of the American Society for Pharmacology and Experimental Therapeutics in recognition of the landmark work that Nebert has accomplished on the pharmacogenetics of the cytochrome P-450 system. We have also been honored by the choice of Dr. Igor Dawid (Chief, Laboratory of Molecular Genetics) to present the NIH's Annual Mider Lecture, one of only two named lectureships at this Institution. Dr. Heiner Westphal received the Public Health Service's Superior Service Award for his exceptional productivity in studies on the transgenic mouse. Dr. David Rodbard received the Public Health Service's Meritorious Service Medal for his development of algorithms that have improved the management of diabetes and other chronic illnesses, particularly in young patients. Dr. Richard Klausner also received the Meritorious Service Medal for having provided the first comprehensive view of the molecular regulation of iron metabolism, vital to all aspects of cell viability and proliferation. Dr. David Klein received the NIH Director's Award for his body of work on the structure and function of the pineal gland and the regulation of its important biosynthetic pathways. Dr. John Donovan was awarded the PHS's Commendation Medal for his leadership in NIH animal care issues. Additionally, many of the Institute's Senior Investigators held honorary lectureships and visiting professorships during the year; major prizes for research accomplishments were awarded to a number of our scientists by various universities and societies.

Finally, we are currently sponsoring two distinguished senior scientists in the Fogarty Scholars-in-Residence Program, Professor Donald Brown of the Carnegie Institution (Washington) and Professor Itzhak Parnas of the Hebrew University (Jerusalem), who are working in our Laboratories during their sabbaticals.

In December of 1982, this Institute embarked upon an ambitious program designed to improve the quality and quantity of its intramural scientific productivity, even as the rate of growth in research support declined. As one reflection of our success in this regard during the past four years, NICHD intramural scientists this year published twice the number of peer-reviewed original scientific reports, appearing in journals of stature, than had been the case prior to the

beginning of this new direction. Moreover, one out of every two of our post-doctoral scientists is now fully supported by a stipend awarded from a non-NIH source--reflecting, we believe, the current quality and productivity of this Intramural Program.

## CELL BIOLOGY AND METABOLISM BRANCH

- |                 |  |
|-----------------|--|
| Z01 HD 01600-02 | Structure and Function of the Murine T Cell Antigen Receptor<br>Richard D. Klausner, M.D.    |
| Z01 HD 01601-02 | Molecular Aspects of the Regulation of the Human Transferrin Receptor<br>Joe B. Harford, Ph. |
| Z01 HD 01602-02 | Regulation of Intracellular Iron Metabolism<br>Jos van Renswoude, M.D.                       |
| Z01 HD 01603-02 | Membrane Traffic and Organelle Biogenesis<br>Ignacio Sandoval, Ph.D.                         |
| Z01 HD 01604-01 | Interleukin-2 Receptor - Structure, Function, and Regulation<br>Warren J. Leonard, M.D.      |





NICHD Annual Report  
October 1, 1985 to September 30, 1986

Cell Biology and Metabolism Branch

The structure and organization of the Cell Biology and Metabolism Branch became more defined in this first full year of operation. Building continued and an 1800 square foot addition to building 18 was completed providing new laboratory space, equipment space, autoclave room and small animal holding room. This completed our initial building program to house the branch. The branch has continued to explore a variety of issues in cell biology including receptor structure and function, receptor gene regulation, role of receptors in the immune response, organelle development and intracellular protein sorting and human iron metabolism. The clinical program in hereditary hemochromatosis has continued and has been well integrated into the research program of the branch.

Research Program

The organization of the branch described in last year's report has been maintained and is outlined below.

<u>Area</u>		<u>Group</u>	<u>Principal Investigator</u>
A. Receptors of the Immune System	1)	Structure and Function of the T Cell Antigen Receptor	R.D. Klausner
	2)	Interleukin-2 Receptor - Structure, Function and Regulation	W.J. Leonard
B. Iron Metabolism	3)	Regulation of Intracellular Iron Metabolism	R.D. Klausner
	4)	Molecular Aspects of the Human Transferrin Receptor	J.B. Harford
C. Organelle Biology	5)	Membrane Traffic and Organelle Biogenesis	I.V. Sandoval

1) Structure and Function of the T Cell Antigen Receptor

The work of this project can be broken down into four areas:

- 1) receptor structure
- 2) biochemistry of receptor activation
- 3) receptor function in immune disease
- 4) receptor assembly

The description of the T cell antigen receptor complex has been extended with the recognition of a seventh protein (see below). Previously we had described and characterized six different proteins which, due to one being a homodimer, form a complex of seven peptide chains. Each was purified and

polyclonal antibodies raised against all chains. The recently published nucleotide sequence of a murine cDNA clone homologous to the human T3 delta chain was used to design synthetic peptides which were utilized to raise an extremely specific anti-murine  $\delta$  chain antiserum. These immunological tools have allowed much greater access to studies about the biosynthesis, assembly, degradation, distribution and biochemistry of the receptor in any T cell and alleviated the previous constraint of having to utilize T cell clones or hybridomas for which anti-clonotypic antibodies were available. In addition cross reactivity between human and murine chains allowed us to assign correct homologies. This latter approach allowed us to identify the gp26 glycoprotein as the  $\delta$  chain of the murine complex. The development of anti- $\zeta$  chain antibodies allowed the identification of the human homologue of this receptor component following upon the original discovery of this chain in our laboratory.

The biochemistry of receptor activation progressed dramatically over the past year. We recognized that antigen and mitogen leads to the simultaneous activation of two distinct phosphorylation pathways. One involves the activation of protein kinase C via the enhanced metabolism of phosphorylated phosphatidyl inositols. This leads to the serine phosphorylation of the  $\gamma$  chain of the complex. In addition a previously unrecognized component of the complex is phosphorylated on tyrosine. This chain is a 21 kd endoglycosaminidase F resistant protein (p21) with a more acidic pI than the  $\gamma$  chain that exists in the complex as a disulfide linked dimer. It is still unclear whether it is a homo- or heterodimer. The finding of a tyrosine kinase coupled to this receptor places this receptor into that small group of cellular receptors involved in growth and differentiation all of which are coupled to tyrosine phosphorylation events. Both the tyrosine and serine phosphorylations represent true de novo addition of phosphates and not phosphate exchange. Although both phosphorylation pathways are activated upon ligand binding the tyrosine phosphorylation does not require the presence of protein kinase C. Cyclic AMP has long been known to block the immune response. We have demonstrated that cAMP uncouples receptor from both phosphorylation pathways. Protein kinase C antagonizes the effects of cAMP on tyrosine phosphorylation but not PI metabolism thus further complicating the interactions between three kinases in the activation of this receptor. We have recently succeeded in reconstituting tyrosine phosphorylation of the receptor in isolated membranes. This promises to allow us to further characterize and dissect the biochemical events observed in intact cells. The implication of phosphatidyl inositol kinase in the coupling of this receptor to the activation of protein kinase C led us to attempt to characterize and purify this important cellular enzyme. The characteristics of the enzyme has been extensive and a major in road into the complete purification been begun. Currently, the enzyme is one to two thousand fold purified.

Characterization of the phosphorylation events in cloned T cells or antigen-specific T cell hybridomas has been extended to normal T cells and to human T cells. In addition two genetically distinct but clinically identical autoimmune/lymphoproliferative diseases of mice have been studied. These mice provide useful animal models for human autoimmune diseases. Because of the central role of the T cell antigen receptor in T cell activation the possibility that these animals have somehow abnormal receptors was tested. Both of these diseases display the same unusual

features of receptor activation: 1) p21 is spontaneously phosphorylated on tyrosine even in the absence of any added ligand; 2) this takes place in the absence of any detectable serine phosphorylation; and 3) mitogen fails to activate these receptors. This gives us the first clue as to possible biochemical lesions responsible for these diseases.

The question of how the cell assembles a 7 to 9 chain membrane complex of a predictable and unique stoichiometry is a fundamental one in cell biology. This question has been addressed by examining the kinetics and pattern of assembly of the T cell receptor after biosynthesis. Assembly occurs rapidly after biosynthesis. Some of the chains are synthesized in great excess with 95-98% of the molecules that are synthesized being degraded with a half-life of about 30 minutes. Only completely assembled chains survive with a half life of between 12 and 20 hours. The degradation of unassembled or incompletely assembled chains appears to occur in lysosomes. This has suggested a general model of assembly whereby the state of assembly (or lack thereof) determines the intracellular routing of integral membrane proteins.

## 2. Interleukin-2 Receptor - Structure, Function and Regulation

The interleukin-2 (IL-2) receptor is responsible for the proliferation of T cells during the immune response. Understanding how its expression is regulated and how it functions is a central problem in immunology. In addition, this receptor has been implicated in the abnormal proliferation of leukemic T cells. The work of this group can be divided into two areas:

- 1) Transcriptional control elements
- 2) Structure of the high affinity receptor

The regulatory regions located 5' to the structural gene have been cloned and partially characterized. When constructs consisting of these flanking regions are fused to the structural gene for chloramphenicol acetyl transferase several important conclusions can be reached. First the regulatory sequences of this gene is highly sensitive to one or more gene products of the human T cell lymphocytotropic I virus (HTLVI) and expression of the hybrid genes is much greater in HTLVI infected cells. The hybrid genes are responsible to treatment of host cells with the tumor promoter phorbol diesters when the genes are introduced to JURKAT cells. This cell is not infected with HTLVI and the expression of its IL-2 receptor is dependent upon treatment with phorbol esters. Finally in HTLVI infected cells a dramatic rise in expression of the hybrid genes is seen when a far 5' flanking region is removed. This strongly points to a negative regulatory element in this gene. Once interesting regulatory sequences are identified, the next step is the identification of specific binding proteins that interact with these sequences. Early progress has been made in this effort with the identification of proteins which bind to specific regions of the 5' end of this gene.

How the IL-2 receptor works remains a mystery. The nature of the signals generated by the binding of ligand to the receptor are, as yet, undefined. One clue involves the observation that there are two classes of IL-2 receptors as defined by ligand binding. One class representing 90-95% of all binding sites displays affinities in the nM range while the rest display binding affinities in the pM range. The minority high affinity receptors



are entirely responsible for both the endocytosis and activity of the ligand. Thus a critical question has become what defines the biochemistry of this high affinity receptor. Studies using covalent cross linking of radiolabeled IL-2 to intact cells have begun to elucidate this issue. When IL-2 was bound to T cells under high affinity conditions and cross linked it was shown to be interacting with two proteins - the 55 kilodalton receptor chain and a previously unidentified 70 kilodalton chain. Cross linking of ligand to low affinity sites showed that the IL-2 in that situation is only interacting with the 55 kilodalton receptor glycoprotein. Thus the high affinity receptor is likely composed of at least two subunits.

### 3. Regulation of Intracellular Iron Metabolism

The major project of this group over the past year has been to molecularly clone the gene for human ferritin H chain. The previous work from the lab pointed to ferritin as the regulator of intracellular iron distribution. This, in turn, was regulated by iron itself which determines the level of ferritin within the cell. In order to develop the tools to study the underlying mechanisms involved, the full gene encoding a ferritin chain was needed. Previous work had demonstrated that many copies of both ferritin H and L chain genes were scattered throughout the human genome. Many of these are likely to be processed pseudogenes and thus finding an expressed gene required a screening strategy to avoid pseudogenes. Such a strategy was designed and a full length copy of the human H chain ferritin gene isolated. This was sequenced and shown to contain a large (~260 base) 5' untranslated region and three introns. The entire length of the gene is about 3 kb. The gene has been introduced into mouse fibroblasts where it has been shown to be expressed. The reconstituted gene functions normally in the murine cells. It is highly regulated by iron, assembles into normal ferritin spheres and co-assembles with mouse ferritin. The functional gene is localized to chromosome 11. Interestingly, preliminary evidence from in situ hybridization suggests the possibility of a chromosomal duplication event placing a copy of this gene at the HLA locus of chromosome 6. This is the predicted localization of the hereditary hemochromatosis gene. The expression of the cloned gene from human chromosome 11 in murine cells is allowing the identification of the nucleotide sequences involved in iron regulation.

### 4) Molecular Aspects of the Regulation of the Human Transferrin Receptor

The central role that the transferrin receptor plays in cellular iron metabolism has led the work of this group to focus on the transcriptional control elements of the gene for the human receptor. The 5' region of the human gene was cloned and transcriptional elements were identified by constructing hybrid genes in which putative regulatory elements are fused to the structural gene for the bacterial enzyme chloramphenicol acetyl transferase (CAT). Such constructs have led to a number of conclusions. Two distinct elements are required in tandem to observe high levels of gene expression. One exists 5' to the transcription initiation site and the other resides in the first intron, approximately 1000 base pairs downstream of the transcription initiation site. Deletion analysis, linker-scanner mutagenesis and sequencing have begun to elucidate the fine structure of these regions. The upstream regions consists of two elements; one located between bases -78 and -62 contains a homologous sequence to the adenovirus

Ela enhancer element and the other, located between -62 and -33, contains SP1 protein binding site sequences. Each contribute to the function of this region. The downstream site has been localized to less than 30 base pairs and contains another homologue to the adenovirus enhancer element. This 3' control region cannot function in the reverse orientation. The identification of these sequences will allow the characterization of specific DNA binding proteins and of the regions required for iron regulation of the expression of this gene.

#### 5) Membrane Traffic and Organelle Biogenesis

The previous development of monoclonal antibodies directed against integral membrane proteins of different intracellular organelles have provided extremely useful tools for examining the relationships between different organelles, the trafficking between organelles and the biogenesis of organelles. This past year most effort was concentrated on a set of integral membrane proteins of the lysosomes. Biosynthetic pulse-labeling studies were used to examine the post translational processing of these proteins. They are all glycosylated and contain N-linked complex carbohydrate chains with sialic acid. This finding demonstrated that they were efficiently transported through the entire Golgi system and reached the trans Golgi before being targeted to lysosomes. Each lysosomal integral membrane protein (LIMP) is transported through the Golgi at similar rates but are retained in the trans Golgi for variable periods of time. Next an assay was developed to monitor the transport of LIMPs from the Golgi to lysosomes. This assay made use of the very different buoyant densities of Golgi and lysosomes and allowed the clear demonstration of the rapid and efficient delivery of these proteins to lysosomes. Removal of N-linked carbohydrate chains has no effect on the transport of these proteins but dramatically destabilizes the LIMPs in the lysosomes. These tools, and the information generated with them, open up the ability to examine the molecular mechanisms of sorting to lysosomes. This is now being approached by the molecular cloning of the genes encoding these proteins.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01600-02 CBMB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the T Cell Antigen Receptor

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Klausner Head CBMB, NICHD

Others:	L. Samelson	Sr. Staff Fellow	M. Baniyash	Vis. Fellow
	A. Weissman	Med. Staff Fellow	C. Suarez-Quian	Guest Researcher
	J. O'Shea	Sr. Staff Fellow	M. Patel	Guest Worker
	J. Harford	Sr. Investigator	H. Luong	Chemist
	Y. Minami	Vis. Fellow		

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.55

## PROFESSIONAL

5.05

## OTHER

.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The receptor on T cells that binds antigen in the context of cell-surface Ia molecules is being studied in order to understand activation and regulation of the immune response. The approach has been to isolate the antigen receptor and characterize its multiple components. The biochemical events accompany antigen interaction with the receptor have been intensively studied in order to identify the mechanisms of signal transduction in T cells.

The antigen receptor on a murine T cell hybridoma has been immunoprecipitated with a monoclonal antibody that uniquely binds its antigen recognition structure. We have shown that the clonally determined  $\alpha$  and  $\beta$  chains are non-covalently associated with five additional chains ( $\delta$ ,  $\epsilon$ ,  $\gamma$ ,  $\zeta$ , p21), two of which are homodimers ( $\zeta$ , p21). Thus the receptor complex consists of nine chains. To extend our studies, antibodies binding the  $\delta$ ,  $\epsilon$ , and  $\zeta$  chains have been prepared. These reagents have been used to characterize the receptor on normal peripheral T cells and to analyze the biosynthesis and assembly of the components of this multichain structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01601-02 CBMB																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <b>Molecular Aspects of the Regulation of the Human Transferrin Receptor</b>																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: J. B. Harford</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">CBMB, NICHD</td> </tr> <tr> <td colspan="3">Others: R. D. Klausner                      Head                      CBMB, NICHD</td> </tr> <tr> <td>K.K. Rao</td> <td>Visiting Associate</td> <td>CBMB, NICHD</td> </tr> <tr> <td>A. M. Weissman</td> <td>Medical Staff Fellow</td> <td>CBMB, NICHD</td> </tr> <tr> <td>T.A. Rouault</td> <td>Medical Staff Fellow</td> <td>CBMB, NICHD</td> </tr> <tr> <td>J. Casey</td> <td>Guest Worker</td> <td>CBMB, NICHD</td> </tr> <tr> <td>B. Di Jeso</td> <td>Visiting Fellow</td> <td>CBMB, NICHD</td> </tr> </table>			PI: J. B. Harford	Senior Investigator	CBMB, NICHD	Others: R. D. Klausner                      Head                      CBMB, NICHD			K.K. Rao	Visiting Associate	CBMB, NICHD	A. M. Weissman	Medical Staff Fellow	CBMB, NICHD	T.A. Rouault	Medical Staff Fellow	CBMB, NICHD	J. Casey	Guest Worker	CBMB, NICHD	B. Di Jeso	Visiting Fellow	CBMB, NICHD
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Others: R. D. Klausner                      Head                      CBMB, NICHD																							
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B. Di Jeso	Visiting Fellow	CBMB, NICHD																					
COOPERATING UNITS (if any)																							
LAB/BRANCH Cell Biology and Metabolism Branch																							
SECTION Section on Organelle and Receptor Structure and Function																							
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS 4.5	PROFESSIONAL: 4.5	OTHER:																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             Iron is taken into proliferating eukaryotic cells via a high affinity receptor for the serum iron-carrying protein transferrin. The transferrin receptor is an integral membrane glycoprotein for which synthesis, degradation, and dynamics are highly regulated. Iron availability modulates the expression of the transferrin receptor in a number of cell types including K562 cells, a human erythroleukemia line. The rate of receptor biosynthesis has been found to be decreased when iron is provided to cells and to be increased when intracellular iron is chelated. The cellular distribution of the receptor and its rate of degradation have been shown to be affected by exposure of K562 cells to a monoclonal antibody recognizing the human transferrin receptor. Using a cDNA probe for the receptor, clones have been isolated from a human genomic library constructed in lambda bacteriophage. One such genomic clone (termed <math>\lambda</math>TR4) has been characterized as representing the 5' portion of the human transferrin receptor gene including the promoter region, exon 1, and a portion of intron 1. A variety of chimeric constructs have been produced in which regions of <math>\lambda</math>TR4 are linked to the bacterial gene for chloramphenicol acetyl transferase (CAT). By transfecting these CAT constructs into CAT-recipient cells, two regions of <math>\lambda</math>TR4 have been implicated as being important in gene expression.           </p>																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01602-02 CBMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Intracellular Iron Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. D. Klausner Head CBMB, NICHD

Others: J. B. Harford Senior Investigator CBMB, NICHD  
M. Hentze Guest Researcher CBMB, NICHD  
S. Keim Guest Researcher CBMB, NICHD  
E. Mattia Guest Researcher (10/85-12/85) CBMB, NICHD  
T.A. Rouault Medical Staff Fellow CBMB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.85

PROFESSIONAL

2.85

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular basis of intracellular iron metabolism rests with the regulation of expression and assembly of ferritin. In order to understand the detailed mechanisms of this regulation we have cloned the entire functional gene for the heavy (H) chain of human ferritin. This gene has been fully characterized and has been introduced into mouse cells in order to dissect the genetic elements responsible for its regulated expression. In addition cross reacting sequences located near the HLA locus of chromosome 6 have been identified and are currently being cloned. This may provide a link to the genetic basis of hereditary hemochromatosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01603-02 CBMB																					
PERIOD COVERED October 1, 1985 to September 30, 1986																							
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Membrane Traffic and Organelle Biogenesis																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: I. V. Sandoval</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 33%;">CBMB, NICHD</td> </tr> <tr> <td colspan="3" style="padding-top: 10px;">Others:</td> </tr> <tr> <td>R. D. Klausner</td> <td>Head</td> <td>CBMB, NICHD</td> </tr> <tr> <td>J. S. Bonifacino</td> <td>Visiting Associate</td> <td>CBMB, NICHD</td> </tr> <tr> <td>C. Suarez-Quian</td> <td>Guest Worker</td> <td>CBMB, NICHD</td> </tr> <tr> <td>J. G. Barriocanal</td> <td>Guest Worker</td> <td>CBMB, NICHD</td> </tr> <tr> <td>L. C. Yuan</td> <td>Chemist</td> <td>CBMB, NICHD</td> </tr> </table>			PI: I. V. Sandoval	Visiting Scientist	CBMB, NICHD	Others:			R. D. Klausner	Head	CBMB, NICHD	J. S. Bonifacino	Visiting Associate	CBMB, NICHD	C. Suarez-Quian	Guest Worker	CBMB, NICHD	J. G. Barriocanal	Guest Worker	CBMB, NICHD	L. C. Yuan	Chemist	CBMB, NICHD
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J. G. Barriocanal	Guest Worker	CBMB, NICHD																					
L. C. Yuan	Chemist	CBMB, NICHD																					
COOPERATING UNITS (if any)																							
LAB/BRANCH Cell Biology and Metabolism Branch																							
SECTION Section on Organelle and Receptor Structure and Function																							
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 4.75	PROFESSIONAL: 3.75	OTHER: 1.0																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues      X <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             Monoclonal antibodies against integral membrane proteins from lysosomes (LIMPs) have been developed to study the biogenesis, turnover and intracellular traffic of these organelles and the problems of sorting implicated in these processes. The results indicate that LIMPs move from the site of their synthesis in the endoplasmic reticulum to the Golgi system. In this organelle they are glycosylated, acquiring N-linked chains of high mannose and complex carbohydrates while moving through the stack of cisternae in a cis to trans direction with similar rates (30 min). Sorting the delivery of LIMPs to lysosomes occurs from the trans-part of the Golgi system. The process of delivery is not synchronous due to the different times of retention of LIMPs in the trans-Golgi (0-90 min). Once their delivery is produced LIMPs are transported to high density lysosomes specifically, efficiently and rapidly (30 min). Transport involves the budding of small size vesicles loaded with LIMPs (i.e. primary lysosomes) from the tubules of the trans-reticular Golgi. LIMPs are transported to the same lysosomes. Neither the sorting nor the delivery of LIMPs is dependent on N-linked carbohydrates and occurs in the presence of tunicamycin. Carbohydrates play an important role in protecting LIMPs against degradation by proteolytic enzymes in lysosomes, as shown by the fast degradation of LIMPs produced in the presence of tunicamycin. Fully glycosylated LIMPs display different half lives in lysosomes.           </p>																							



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01604-01 CBMB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Interleukin-2 Receptor - Structure, Function, and Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Warren J. Leonard Senior Staff Fellow CBMB, NICHD

Others: Sharon L. Cross Guest Researcher CBMB, NICHD  
 Richard D. Klausner Chief CBMB, NICHD  
 Myra Lipas Medical Staff Fellow CBMB, NICHD  
 Michael Sharon Medical Staff Fellow CBMB, NICHD  
 Julie B. Wolf Guest Technician CBMB, NICHD

## COOPERATING UNITS (if any)

Nancy Chang Baylor College of Medicine, Houston, TX  
 Richard Chizzonite Hoffmann La Roche, Inc., Nutley, NJ  
 Matija Peterlin HHMI, Univ. of Calif. San Francisco, CA

## LAB/BRANCH

Cell Biology and Metabolism Branch

## SECTION

Section on Organelle and Receptor Structure and Function

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

5.1

## PROFESSIONAL:

4.1

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human interleukin-2 receptor (IL2R) is being studied in order to understand specific critical components of the T cell immune response in both normal and neoplastic cells. The approaches used are based on (1) identification of key transcription regulatory sequences in the IL2R gene; (2) identification of DNA binding proteins for the promoter region; (3) biochemical analysis of the distinction between high and low affinity IL2Rs.

Using IL2R cDNA and genomic constructs, we have partially mapped the areas of the IL2R gene necessary for transcriptional activity, as determined by the ability to drive expression of the bacterial chloramphenicol acetyltransferase gene following transfection of human cells. In MT-2 and HUT-102 cells (both infected with HTLV-I), promoter activity appears to increase if one removes regions more than 347 bases 5' to the cap site, suggesting the possibility of a negative regulatory element. This effect is less dramatically seen in JURKAT cells. Promoter activity is lost if one removes all regions more than 244 bases 5' to the cap site in MT-2 and HUT-102 cells or more than 266 bases 5' to the cap site in JURKAT cells. We have also identified a putative DNA binding protein in nuclear extracts. This protein has been partially purified by heparin agarose chromatography.

In order to study the biochemical distinction between high and low affinity IL2Rs, we have cross-linked <sup>125</sup>I-IL2 (M<sub>r</sub> 15,500) to the IL2R (M<sub>r</sub> 50-55,000). In addition to the expected band of 68-72 kD we find a second band of 85-92 kD. Immunoprecipitations with anti-IL2R antibodies only precipitate the 68-72 kD band. We believe that the upper band represents IL-2 cross-linked to a high affinity IL2R specific associated subunit, which confers high affinity binding properties to the IL2R.





DEVELOPMENTAL ENDOCRINOLOGY BRANCH

- Z01 HD 00610-06 Puberty and its Disorders: Physiology, Pathophysiology  
and Therapy  
Gordon B. Cutler, Jr., M.D.
- Z01 HD 00613-06 Clinical and Basic Studies of Male Reproduction  
Richard J. Sherins, M.D.
- Z01 HD 00614-06 Biology of Hormone Binding Proteins  
Bruce C. Nisula, M.D.
- Z01 HD 00615-06 Steroid Antagonists  
George P. Chrousos, M.D.
- Z01 HD 00616-06 Structure, Function, and Physiology of Glycoprotein  
Hormones  
Bruce C. Nisula, M.D.
- Z01 HD 00618-05 Physiology and Pathophysiology of Stress  
George B. Chrousos, M.D.
- Z01 HD 00619-05 Hypothalamic-Pituitary-Gonadal Interaction  
D. Lynn Loriaux, M.D.
- Z01 HD 00621-04 Mechanism of Linear Growth  
Fernando Cassorla, M.D.
- Z01 HD 00622-04 Diagnostic and Therapeutic Applications of  
Growth Hormone Releasing Factors  
George R. Merriam, M.D.
- Z01 HD 00623-03 Adrenal Physiology and Pathophysiology  
Gordon B. Cutler, Jr., M.D.



Developmental Endocrinology Branch

The research aim of the Developmental Endocrinology Branch is to further our understanding of the role of the endocrine system in the complex processes of growth, development and reproduction. The endocrine system is being studied in fetal and neonatal life, childhood, young adulthood and old age. Most of our current research is focused on the pubertal transition. Systems under study include the hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-thyroid axis, the systems regulating growth, and placental fetal interactions. The following summary will highlight the past year's research accomplishments. It is not intended to be a complete compendium of the year's research activities. This can be obtained from the individual project reports.

Studies on Growth

The primary focus of our current studies on growth is to understand the relative roles of the steroid hormones and the various growth factors in regulating skeletal growth and epiphyseal maturation.

One of the critical problems in the study of growth is that the process is slow. This translates into difficulty in making reliable measurements over short periods of time. We have sought to solve this problem by using devices that measure the length of a single bone or limb segment with great precision. To examine the performance of these assays we measured stature and the lower leg length of 13 normal prepubertal children at three-week intervals for one year. Height velocity at three-week intervals did not show significant differences across the year. At six- and twelve-week intervals, spring and autumn growth velocity was higher. The amplitude of the variations however, was small. These data suggest that, contrary to earlier dogma, only minimal variations in growth velocity occur during the year. There is no evidence of a major seasonal influence on growth. Using this methodology, we have begun to study the mechanism of pubertal growth. The primary objective is to define the relative roles of the steroid sex hormones, growth hormone, and the growth factors (IGF-I, IGF-II).

To evaluate the hypothesis that sex steroids increase spontaneous growth hormone secretion, we measured GH secretion and GH responses to standard provocative tests in twenty-one children with idiopathic short stature. Eight of these children were pretreated with sex hormone (estrogens for girls, androgens for boys). All children were prepubertal and had no endocrine abnormalities. The children who received sex steroid pretreatment showed a greater frequency of growth hormone secretory bursts ( $6.0 \pm 0.3$  (SEM) vs.  $3.2 \pm 0.3$ ,  $p < 0.02$ ). The peak responses of growth hormone to arginine and to L-dopa were higher in sex hormone treated children ( $27.3 \pm 5.5$  ng/ml,  $p < 0.02$  vs.  $9.8 \pm 1.9$ , and  $11.4 \pm 3.3$  vs.  $5.7 \pm 2.2$ , respectively). Responses to insulin and to GHRH, however, were not different between the two groups. This data supports the concept that sex steroids stimulate growth hormone secretion, and is compatible with the idea that sex steroid influence growth, at least in part, through altering secretion of growth hormone. The dose response relationship between sex hormone and growth

was examined in children with Turner's syndrome. Previous studies showed that the dose of ethinyl estradiol yielding optimum growth in girls with Turner's syndrome was 10 ng/kg/d. This is considerably lower than the dose currently recommended by most textbooks. We have now examined the effects of low-dose estrogen treatment over a 6-month period. Growth was sustained without accelerating bone age advancement. This suggests that ultimate stature in patients with Turner's syndrome may be improved with low-dose estrogen treatment. These data also imply that the currently recommended regimen may be harmful and actually reduce ultimate height. These findings represent an important advance in the treatment of Turner's syndrome, a disorder characterized by ultimate short stature.

The role of sex steroid in pubertal growth has also been examined by reducing sex steroid secretion in children with precocious puberty using a long action analogue of LHRH. We have examined the results of 2 to 4 years of such therapy in 27 children with central precocious puberty. Linear growth rates decreased from  $11.0 \pm 0.8$  cm/yr before treatment to  $5.7 \pm 0.4$  cm/yr at two years of treatment ( $p < 0.025$ ). Predicted heights by the Bayley-Pinneau method increased from  $156.4 \pm 2.0$  cm before treatment to  $162.3 \pm 2.3$  cm at two years ( $p < 0.001$ ). Five patients treated for 4 years had a mean increase in predicted height of 5.5 cm. No adverse effects have been observed. This study demonstrates sustained effectiveness of D-Trp<sup>6</sup>-Pro<sup>9</sup>-NET-LHRH in central precocious puberty over a period of 2 to 4 years. Growth rate and bone maturation slowed and predicted height increased by 5-7 cm over the first 2-4 years. LHRHa thus appears to constitute an effective long term therapy for precocious puberty. Its effects on growth are mediated by changes in sex steroid hormones.

The role of growth hormone itself has been explored by altering its concentration with growth hormone releasing hormone (GHRH). An additional goal of these studies has been to determine whether or not GHRH will be an effective therapeutic agent in growth hormone deficiency (GHD). GHRH has previously been shown to accelerate growth in children. GHRH administration by infusion pump or by i.v. injection, however, is demanding and labor-intensive. Subcutaneous administration certainly would be simpler, but the relative efficacy of this approach is unknown. We examined this issue by treating 7 prepubertal GHD children with GHRH, given subcutaneously by pulsatile infusion pumps at 3 ug/kg/pulse or with intermittent subcutaneous injections three or four times a day. Subjects underwent several successive 6-week treatment periods with 3-week intervals between. The pumps delivered 0-16 pulses/d, and were used only at night. Doses ranged from 1 mcg/kg/dg to 27 mcg/leg/day. Height and lower leg measurements were made at 3-week intervals. Both treatments stimulated GH secretion. Both accelerated lower leg growth velocities. Thus, it is not necessary to normalize the pattern of growth hormone secretion to stimulate growth, and once a day subcutaneous injection regimens of GHRH can be an effective treatment for children with growth hormone deficiency.

#### Studies on the Disorders of Puberty

One of our primary research objectives is to understand the physiologic processes underlying the initiation of puberty. As part of this effort, we have examined several clinical disorders of puberty. The McCune-Albright syndrome, for example, consists of a triad which includes precocious puberty, polyostotic fibrous dysplasia and café au lait pigmentation of the skin. The gonadal activity of these



patients seems to be independent of pituitary gonadotropin secretion. This finding led to the hypothesis that some as yet unidentified circulating factor was responsible for this gonadal activation in this syndrome.

To test this hypothesis, sera from patients and matched control subjects were tested in a rat Sertoli cell FSH bioassay. The assay measured FSH dependent aromatase activity. McCune-Albright patients had slightly increased FSH bioactivity compared to prepubertal controls but the level was too low to explain the degree of estradiol elevation seen in this disorder. Patients with familial male precocious puberty were examined in an analogous way, except that LH activity was searched for. Sera from 7 patients and from 6 prepubertal control children, matched for gonadotropin level by RIA, were studied. Testosterone secretion exceeded the upper limit for the control samples in four. Thus, testis activation in this disorder may be caused by a circulating factor that is not detected by gonadotropin radioimmunoassay. These studies provide new insight into the mechanisms of gonadotropin independent precocious puberty and provide a factual foundation upon which rational therapy can be based. For example, patients with the McCune-Albright syndrome have been treated successfully with testolactone, an aromatase inhibitor, and patients with familial male precocious puberty have been treated with a combination of testolactone and an antiandrogen. The antiandrogen (spironolactone) decreased androgenic manifestations of precocious puberty, such as acne and spontaneous erections, but did not control accelerated growth and bone maturation. Testolactone normalized growth rate and the rate of bone maturation.

#### Hormone Transport and Action

The disorders and physiologic processes outlined above are entrained to the action of the steroid and glycoprotein hormones. In an attempt to increase our understanding of this process we have examined the relationship between hormone structure and subsequent function using the glycoprotein hormones and binding globulins as models.

It has been found that the alpha-subunit derived from intact hCG contains oligosaccharide side chains that are primarily terminated in a single sialic acid (i.e., monosialylated). This was unexpected since the structure of the oligosaccharides was believed to be bifurcated, both chains ending in a sialic acid residue (i.e., disialylated). Apart from the value of this finding to structural biochemistry it contributes to a growing body of evidence supporting a role for the carbohydrate of hCG in receptor binding, target cell activation, metabolism, and combination with complimentary subunits. In contrast, most of the free alpha-subunit (subunits not derived from intact hCG) oligosaccharides are di- and trisialylated. Since the more heavily glycosylated free alpha-subunits are incapable of combining with native hCG beta-subunit to form the intact hormone, it is possible that the carbohydrate on free alpha-subunit serves to regulate net hCG synthesis by preventing combination with the hCG beta-subunit. These studies have opened new areas of investigation into the regulation of hCG biosynthesis.

Previous studies of the thyrotropic activity intrinsic to hCG showed that removal of its carbohydrate converts hCG from an agonist to an antagonist of TSH action. Thus, we hypothesized that the carbohydrate moieties of TSH would also play a critical role in the activity of TSH with its receptor. This has proved to be



the case. Studies of the functional properties of the deglycosylated TSH were performed using human thyroid membranes. Deglycosylated TSH bound to TSH receptors in thyroid membranes with more than twice the affinity of native TSH. Despite its enhanced binding affinity, however, deglycosylated TSH was able to stimulate only 10% of the activity of the adenylate cyclase activity achieved with native TSH. The deglycosylated TSH also exhibited antagonistic activity toward TSH stimulated adenylate cyclase activity. These findings provide the first direct evidence for the role of the TSH carbohydrate moieties in the transduction of the TSH hormonal signal across the thyroid cell membrane. From the clinical perspective, these data provide a rational basis for the development of a TSH antagonist that could be used to treat patients with hyperthyroidism.

In healthy human subjects TSH, is secreted in a circadian pattern. The lowest level occurs at about 5:00 in the afternoon and the peak occurs between midnight and 4:00 in the morning. The progressive rise in the serum TSH concentration from 5:00 to midnight is called the "nocturnal TSH surge." Thyroid gland function exhibits a similar pattern. The nocturnal TSH surge is presumably related to a signal generator in the central nervous system, like many other biologic rhythms. Awareness of this phenomenon prompted us to predict that certain diseases of the central nervous system might interfere with the signals that mediate the nocturnal surge and thus lead to hypothyroidism. The nocturnal surge of TSH was examined in a series of 16 patients with hypothalamic and/or pituitary disease. Six of the patients were hypothyroid. The nocturnal TSH surge was absent in the 6 hypothyroid patients and present in 10 euthyroid patients. The significance of this finding derives from its clarification of the mechanism for hypothyroidism associated with hypothalamic disease, and its potential use as a clinical test for patients with hypothalamic and/or pituitary disease who are at risk for developing central hypothyroidism.

The total amount of hormone in plasma often does not reflect its bioavailability. This is because only the "free hormone" (unbound hormone) appears to be available to the tissues. Accurate measurement of the free hormone has been impeded by the unavailability of ultrapure radioligands. The impurities, often only a small fraction of the total radioactivity, produce large errors in the measurement of free steroid hormone. We have devised a practical procedure to separate radioactive impurities from steroid radioligands. The technique employs a solid phase of Sepharose-Con A-binding globulin to precipitate the steroid of interest, non-binding species are excluded, purify the binding species of interest. This improved assay system has been used to study the binding globulins in a number of disorders. The levels of most binding globulins are regulated by specific physiologic, ontologic and endocrinologic factors; for example, a rapid dose-related response of sex hormone binding globulin (SHBG) to treatment with thyroxine was demonstrated in familial dysalbuminemic hyperthyroxinemia. This was the first demonstration of thyroid hormone responsivity in patients with this condition. Evidence that the adrenal axis modulates SHBG and cortisol binding globulin (CBG) levels has also been found. The levels of both these steroid binding proteins increases in patients with Cushing's disease during blockade of glucocorticoid action with the receptor antagonist RU 486. This observation suggests that levels of these proteins in plasma may serve as measures of response to therapy and provide new tools to assess the adequacy of several commonly used treatment regimens for glucocorticoid excess.

factor are potential candidates. To this end, we have shown that epidermal growth factor stimulates ACTH secretion in primate pituitary cells in vitro at doses or concentrations equimolar to those of CRF. It remains to be shown if this phenomenon has physiologic relevance.

In the search for tissue CRF(s) we have examined two models of stress. The first is surgery. Patients undergoing neck exploration were examined before, during and after surgery. We were surprised to see only mild activation of the HPA axis and the adrenomedullary system during surgery. ACTH and cortisol secretion was continuous rather than pulsatile, the bulk of ACTH and epinephrine secretion taking place during anesthesia reversal.

The second is exercise. Exercise is a component of the "fight or flight" response and is known to be activated in an intensity-dependent fashion that correlates well with both percent maximal  $O_2$  consumption ( $VO_{2max}$ ) and plasma lactate elevation. We used a treadmill to quantitate exercise. Trained subjects could perform with minimal activation of the axis, seemingly the result of chronic adaptation to exercise stress. This was not true for the sedentary subjects. There was, in all subjects, a direct relationship between exercise intensity and activities of the HPA axis. Lactate appears to be a good candidate for the messenger molecule. Lactate stimulates pituitary ACTH secretion in vitro and pyruvate does not. Studies that test the action of L-lactate as a CRF in vitro are now in progress. These studies show that exercise and stress go hand in hand, and that the stress response can be controlled by training.

#### Studies on Reproduction

Studies on reproduction during the last year have concentrated on the infertile male. Attention has been focused on factors that could explain idiopathic infertility. We have shown that the motility of sperm from infertile men is the same as that from normal matched controls, suggesting that sperm motion is not an important variable. Other factors however, do seem to differentiate the infertile population from normal. These include the distribution of epididymal proteins over the sperm head and the response of the sperm to hyposmotic shock. The biochemical abnormalities underlying these phenomena are unknown, but clarification of these issues holds promise for rational therapy.

The treatment of hypogonadotropic hypogonadism has been traditionally based on replacement therapy with hCG and FSH. Recent studies in other centers have proposed that LHRH given in a pulsatile fashion from an external pump may be superior to the standard regimen of gonadotropins. We have explored this concept with a dose response study of LHRH against testis size and sperm count. The results show that testis size is improved by the pump compared to the standard regimen, but that sperm counts are not different. Since pump treatment is exceedingly labor intensive, it seems that the traditional treatment may be the preferred one at the present time.



## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-06 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Puberty and its Disorders: Physiology, Pathophysiology and Therapy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI: Gordon B. Cutler, Jr.

Head

DEB, NICHD

Others: (see attached list)

## COOPERATING UNITS (if any)

National Institute of Mental Health; Child and Family Research Branch, NICHD;  
(see attached list)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Section on Developmental Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

6.4

## PROFESSIONAL

5.6

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

The objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Principal areas of investigation include the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone releasing hormone, the treatment of the McCune-Albright syndrome with an aromatase inhibitor, and the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor.



Others:	D. L. Loriaux	Chief	DEB, NICHD
	B. Albertson	Staff Fellow	DEB, NICHD
	K. M. Barnes	Chemist (Tech)	DEB, NICHD
	F. Cassorla	Senior Investigator	DEB, NICHD
	G. Chrousos	Senior Investigator	DEB, NICHD
	G. Daniel	Guest Researcher (Intramural NRSA)	DEB, NICHD
	P. Feuillan	Med. Staff Fellow	DEB, NICHD
	L. Laue	Med. Staff Fellow	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	P. Manasco	Med. Staff Fellow	DEB, NICHD
	M. Nicoletti	Visiting Fellow	DEB, NICHD
	S. Rose	Med. Staff Fellow	DEB, NICHD
	J. Levine Ross	Guest Researcher	DEB, NICHD
	M. Uriarte	Visiting Associate	DEB, NICHD
	R. Whitcomb	Med. Staff Fellow	DEB, NICHD

#### Cooperating Units:

LDP, National Institute of Mental Health (E. Susman, E. Nottelmann, G. Inoff, L. Dorn, J. Blue); Child and Family Research Branch, NICHD, NIH (R. Klein); Clinical Center, NIH (M. Skerda, A. McNemar, K. Hench, A. Dwyer, T. Shawker M. Platia); Department of Pediatrics, Yale Univ. (F. Comite); Department of Obstetrics and Gynecology, SUNY at Buffalo (A. Munabi); Department of Child Psychiatry, University of Minnesota (W. Sonis); Department of Pediatrics, University of Michigan (C. Foster); Department of Obstetrics and Gynecology, SUNY at Stony Brook (D. Kenigsberg); Department of Radiology, Fairfax Hospital (K. Rieth); National Institute of Dental Research (M. Roberts); Department of Pediatrics, Johns Hopkins University (C. Van Dop); Birth Defects Branch, Center for Disease Control (G. Oakley); Department of Pediatrics, University of Minnesota (O. Pescovitz); Department of Internal Medicine, McMaster University Medical Centre (J. Booth); Department Internal Medicine, University of Dalhousie (R. Rittmaster).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00613-06 DEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Clinical and Basic Studies of Male Reproduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R.J. Sherins Head DEB, NICHD

Others: (see attached list)

COOPERATING UNITS (if any)

(see attached list)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Reproductive Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

6.0

PROFESSIONAL

6.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The objectives of this study are to ascertain biological, physiological and clinical mechanisms of male reproductive disorders and to provide rational strategies of treatment for men with reproductive disease.

This project represents a continuum of research begun in 1970 and includes studies of 1) the hormonal regulation of spermatogenesis in gonadotropin deficient men, 2) biology of sperm function, 3) adverse effects of cancer therapy on gonadal function, 4) evaluation of treatment of men with reproductive disorders and 5) the role of sex steroids in regulation of gonadotropin secretion.

Major findings from studies performed during the past year have shown 1) that pulsatile GNRH does not improve sperm production over that achieved with exogenous gonadotropins in hypogonadotropic men, 2) that sperm of infertile men show a pattern of tail swelling after exposure to hypoosmotic medium that distinguishes it from the pattern seen in sperm of normal men, 3) that in using automated computer assisted semen analysis the quality of sperm motility in men with unexplained infertility is indistinguishable from that of normal fertile men, and 4) that estradiol, administered at physiologic dosage to castrated male rats, increases in-vitro synthesis and secretion of LH  $\alpha$ , LH  $\beta$  and free LH  $\alpha$  subunits as well as prolactin synthesis; effects opposite to those of testosterone.

## Others:

D.L. Loriaux	Chief	DEB, NICHD
G.B. Cutler	Senior Investigator	DEB, NICHD
B.C. Nisula	Senior Investigator	DEB, NICHD
F. Cassorla	Senior Investigator	DEB, NICHD
L. Liu	Medical Staff Fellow	DEB, NICHD
A. Burris	Medical Staff Fellow	DEB, NICHD
S. Rose	Medical Staff Fellow	DEB, NICHD
D. Vogel	Guest Researcher (NRSA)	DEB, NICHD
G. Daniel	Guest Researcher (NRSA)	DEB, NICHD
D. Vantman	Guest Researcher	DEB, NICHD
J. Davidson	Guest Researcher	DEB, NICHD
S. Carter	Guest Researcher	DEB, NICHD
J. Blaquier	Guest Researcher	DEB, NICHD
G. Merriam	Guest Researcher	DEB, NICHD
R. Clark	Guest Researcher	DEB, NICHD
J. Booth	Guest Researcher	DEB, NICHD

## Cooperating Units:

T. Kinsella	ROB, NCI
M. Lippman	DCT, NCI
R. Makuch	DMB, NCI
R. Fischell	APL, Johns Hopkins University
B. Weintraub	CEB, NIADDK
S. Carter	University of Maryland
A. Yergy	LTPB, NICHD

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00614-06 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Biology of Hormone Binding Proteins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

PI:	B. C. Nisula	Head	DEB, NICHD
Others:	G. Chrousos	Visiting Scientist	DEB, NICHD
	R. Hiramatsu	Visiting Fellow	DEB, NICHD
	D. Loriaux	Head, SSH	DEB, NICHD
	L. Nieman	Med. Staff Fellow	DEB, NICHD
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Medical Endocrinology Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.3

## PROFESSIONAL

1.1

## OTHER

.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduceo type Do not exceed the space provided )

The general goals of this project are to understand the biology of the circulating hormone binding proteins and to delineate the role that they play in human disease. In the current period, we have developed a new method for preparation of radioligands for use in determination of free steroid hormone levels in human plasma. The importance of this methodological advance lies in the fact that the free fraction of plasma hormone closely reflects the portion of hormone that is available to the tissues, which is a key clinical diagnostic measure. In addition, we have made progress in expanding the clinical applications of binding proteins for steroid hormones. We found plasma sex hormone-binding globulin measurements useful in demonstrating thyroid hormone responsiveness in familial dysalbuminemic hyperthyroxinemia. Novel evidence of the role of glucocorticoids in regulating both sex hormone-binding globulin and corticosteroid-binding globulin levels in plasma was uncovered in patients with Cushing's syndrome using a glucocorticoid antagonist. Our future research effort will be directed toward developing techniques for assessing intracellular hormone levels using blood specimens obtained in the clinic and correlating this measure with parameters of hormone transport or action in various clinical conditions, especially pregnancy.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00615-J6 DFB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Steroid Antagonists

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	G.P. Chrousos	Head	DEB, NICHD	
Others:	D. D. Brandon	Chemist	DEB, NICHD	Guest Staff:
	G. B. Cutler, Jr	Head, DES	DEB, NICHD	S. Kawai
	L. Golden	Medical Staff Fellow	DEB, NICHD	T. Loughlin
	L. Laue	Medical Staff Fellow	DEB, NICHD	G. Merriam
	D. L. Loriaux	Chief	DEB, NICHD	
	L. Nieman	Medical Staff Fellow	DEB, NICHD	
	R. Udelsman	Medical Staff Fellow	DEB, NICHD	

## COOPERATING UNITS (if any)

Division of Veterinary Resources (M. Morin)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Unit on Hypothalamic Releasing Factors

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

## TOTAL MAN-YEARS

2.2

## PROFESSIONAL

2.0

## OTHER

0.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. Antagonists for the glucocorticoids or the progestins with potential clinical usefulness have been discovered only recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids.

Initially, we proved that glucocorticoid antagonists can be developed by modifications of the 11-position of the steroidal C ring of glucocorticoids. Then we tested a prototype glucocorticoid-progestin antagonist (RU 486) developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals.

Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that anti-glucocorticoids could be used for challenging the hypothalamic-pituitary-adrenal axis when clinical testing is required in patients with disorders of this axis. Antiglucocorticoid therapy of patients with severe Cushing's syndrome due to ectopic ACTH secretion or adrenocortical tumors causes remission of the clinical manifestations of hypercortisolism. We have treated 7 patients and are currently enlarging the therapy series, including patients with Cushing's syndrome prepared for major surgery.

Given to women in single monthly doses during the luteal phase of the cycle RU 486 causes vaginal bleeding. The subsequent cycle is of normal duration. This suggests that single doses of RU 486 could be used for contraception.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00616-06 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure, Function, and Physiology of Glycoprotein Hormones

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. C. Nisula	Head	DEB, NICHD
Others:	D. Blithe	Staff Fellow	DEB, NICHD
	L. Liu	Med. Staff Fellow	DEB, NICHD
	S. Rose	Med. Staff Fellow	DEB, NICHD
	R. Wehmann	Special Expert	DEB, NICHD
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Medical Endocrinology Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.7

## PROFESSIONAL:

2.9

## OTHER:

.8

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The overall objectives of this project are to understand the endocrinology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances include the following: Identification of novel molecular structures in the oligosaccharide moieties of the human choriogonadotropin and free alpha-subunit of pregnancy; demonstration of a deficiency in the normal physiological pattern of thyrotropin secretion in patients with hypothalamic and/or pituitary diseases; and demonstration that the oligosaccharide moieties of thyrotropin play an essential role in the activation of the adenylate cyclase second messenger system. Future directions of the project will include assessment of the functional impact of proteolytic digestion of the choriogonadotropin subunits, elucidation of renal mechanisms for catabolism of choriogonadotropin and related molecules, exploration of naturally-occurring antagonists to follicle-stimulating hormone, and further characterizations of the carbohydrate structure of choriogonadotropin and its subunits in pregnancy.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00618-05 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Physiology and Pathophysiology of Stress

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G.P. Chrousos

Head

DEB, NICHD

Others: (see attached list)

COOPERATING UNITS (if any) CNS, BPB, NIMH (P. Gold); CNS Section, SNB, NINCDS (E. Oldfield); LDP, NIMH (E. Sussman, E. Nottelman, G. Inoff), LCP, NIA (M. Blackman, E. Pavlov, M. Harmon), EPL, Dept of Military Medicine, USUHS (Patricia Deuster)., Dept. of Pediatrics, Temple University (J. Levine-Ross).

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Unit on Hypothalamic Releasing Factors

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.2

## PROFESSIONAL

2.6

## OTHER

0.6

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

In this project we seek to advance the understanding of the physiology and pathophysiology of stress. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas:

- 1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome and pseudo-Cushing's syndrome (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, depression and anorexia nervosa.
- 2) Regulation of the hypothalamic-pituitary-adrenal axis in vivo and in vitro: The regulation of the axis by opioids, vasopressin, oxytocin and glucocorticoids has been studied in vivo. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has been examined in vitro in a newly established hypothalamic organ culture system. Athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Maternal dexamethasone at replacement doses suppresses the fetal adrenal. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents.
- 3) Role and actions of glucocorticoids: The effects of glucocorticoids upon the cardiovascular system during surgical stress are merely permissive. Glucocorticoid resistance is associated with normal size glucocorticoid receptor subunits that have decreased affinity for glucocorticoids.

Others:	D. D. Brandon	Chemist	DEB, NICHD
	A. Calogero	Visiting Fellow	DEB, NICHD
	G. B. Cutler, Jr.	Head, DES	DEB, NICHD
	M. Grino	Visiting Fellow	DEB, NICHD
	L. Laue	Medical Staff Fellow	DEB, NICHD
	D. L. Loriaux	Chief	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	R. Udelsman	Medical Staff Fellow	DEB, NICHD

## Full Time Guest Researcher Staff:

P. Avgerinos	Guest Researcher	DEB, NICHD
W. Gallucci	Guest Researcher	DEB, NICHD
S. Kawai	Guest Researcher	DEB, NICHD
A. Luger	Guest Researcher	DEB, NICHD
A. Margioris	Guest Researcher	DEB, NICHD
A. Markwick	Guest Researcher	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00619-05 DEB																
PERIOD COVERED October 1, 1985, to September 30, 1985																		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Hypothalamic-Pituitary-Gonadal Interaction																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I.</td> <td style="width: 35%;">D.L. Loriaux</td> <td style="width: 35%;">Head</td> <td style="width: 15%;">DEB, NICHD</td> </tr> <tr> <td>Others:</td> <td>G.R. Merriam</td> <td>Junior Investigator</td> <td>DEB, NICHD</td> </tr> <tr> <td></td> <td>L. Nieman</td> <td>Medical Staff Fellow</td> <td>DEB, NICHD</td> </tr> <tr> <td></td> <td>B. Albertson</td> <td>Staff Fellow</td> <td>DEB, NICHD</td> </tr> </table>			P.I.	D.L. Loriaux	Head	DEB, NICHD	Others:	G.R. Merriam	Junior Investigator	DEB, NICHD		L. Nieman	Medical Staff Fellow	DEB, NICHD		B. Albertson	Staff Fellow	DEB, NICHD
P.I.	D.L. Loriaux	Head	DEB, NICHD															
Others:	G.R. Merriam	Junior Investigator	DEB, NICHD															
	L. Nieman	Medical Staff Fellow	DEB, NICHD															
	B. Albertson	Staff Fellow	DEB, NICHD															
COOPERATING UNITS (if any)  P. Platia, Medical Staff Fellow, CC, NICHD Roussel-UCLAF (Dr. E.E. Baulieu), Paris France, Dept. Radiology, Clinical Center, NIH																		
LAB/BRANCH Developmental Endocrinology Branch																		
SECTION Section on Steroid Hormones																		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892																		
TOTAL MAN-YEARS  1.63	PROFESSIONAL  1.63	OTHER  0																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )  Studies this year have centered on the pathophysiology of Leydig cell activation in patients with familial precocious puberty. This interesting group of patients demonstrate what appears to be gonadotropin independent Leydig cell activity. Previous studies have focussed on the possibility that a humoral factor is responsible for this process. Several groups have searched for this hypothetical factor using in vitro assays of Leydig cell function, usually the dispersed rat Leydig cell assay. These systems have failed to reveal Leydig cell stimulators. We reasoned that the heterologous nature of the assay system might explain the failure to detect such substances. We examined this hypothesis by infusing plasma from patients with the disorder and from pubertal stage matched controls directly into the spermatic artery of rhesus monkeys. Testosterone secretion into the testicular vein served as the response parameter. This system separated the precocious puberty group from the other two, supporting the hypothesis that a circulating factor may play a role in the pathogenesis of this syndrome.																		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00621-04 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Linear Growth

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	F. Cassorla	Visiting Scientist	DEB, NICHD
Others:	G. B. Cutler, Jr.	Head	DEB, NICHD
	G. R. Merriam	Guest Worker	DEB, NICHD
	S. Rose	Medical Staff Fellow	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	S. G. Ren	Guest Worker	DEB, NICHD
	M. Nicoletti	Visiting Fellow	DEB, NICHD
	D. L. Loriaux	Chief	DEB, NICHD

## COOPERATING UNITS (if any)

Clinical Center, NIH (M. Skerda); Metabolism Branch, NCI (M. Gelato) Catholic University of Nijmegen, The Netherlands (I.M. Valk); Hanemann Medical School, Philadelphia, Pennsylvania (J.L. Ross);

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Section on Developmental Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

## TOTAL MAN-YEARS

1.4

## PROFESSIONAL:

1.4

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include studying short term growth in normal children. In addition, we are investigating the growth of patients with precocious puberty, and the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. We are studying the mechanism of catch up growth in small for gestational age infants. We are also attempting to define the optimal dose of hydrocortisone for growth in patients with adrenal insufficiency. In addition, we are examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double blind, placebo-controlled clinical trial. In addition, we are investigating the growth hormone secretory dynamics in patients with hypophosphatemic rickets. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone deficient children by using different treatment regimens in order to optimize growth.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00622-04 DEB																								
PERIOD COVERED October 1, 1985 to September 30, 1986																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormones</b>																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G. R. Merriam</td> <td style="width: 33%;">Guest Researcher</td> <td style="width: 33%;">DEB, NICHD</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: F. Cassorla</td> <td>Visiting Scientist</td> <td>DEB, NICHD</td> </tr> <tr> <td>M. C. Gelato</td> <td>Guest Researcher</td> <td>DEB, NICHD</td> </tr> <tr> <td>T. Loughlin, M.D.</td> <td>Guest Researcher</td> <td>DEB, NICHD</td> </tr> <tr> <td>D. L. Loriaux</td> <td>Chief</td> <td>DEB, NICHD</td> </tr> <tr> <td>S. Malozowski</td> <td>Visiting Fellow</td> <td>DEB, NICHD</td> </tr> <tr> <td>M. Nicoletti, M.D.</td> <td>Visiting Fellow</td> <td>DEB, NICHD</td> </tr> </table>			PI: G. R. Merriam	Guest Researcher	DEB, NICHD				Others: F. Cassorla	Visiting Scientist	DEB, NICHD	M. C. Gelato	Guest Researcher	DEB, NICHD	T. Loughlin, M.D.	Guest Researcher	DEB, NICHD	D. L. Loriaux	Chief	DEB, NICHD	S. Malozowski	Visiting Fellow	DEB, NICHD	M. Nicoletti, M.D.	Visiting Fellow	DEB, NICHD
PI: G. R. Merriam	Guest Researcher	DEB, NICHD																								
Others: F. Cassorla	Visiting Scientist	DEB, NICHD																								
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S. Malozowski	Visiting Fellow	DEB, NICHD																								
M. Nicoletti, M.D.	Visiting Fellow	DEB, NICHD																								
COOPERATING UNITS (if any) University of Catania, Italy; INTA, Chile; Chinese Academy of Medical Sciences, Beijing; University of Minnesota; Dalhousie University (See Attached)																										
LAB/BRANCH Developmental Endocrinology Branch																										
SECTION Section on Steroid Hormones																										
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS 2.5	PROFESSIONAL: 2.25	OTHER: .25																								
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Growth hormone-releasing hormone (GHRH) is the hypothalamic peptide which stimulates the release and synthesis of growth hormone (GH). The long-term aims of this project are a) to define the role of GHRH in the regulation of GH secretion; b) to study the modulation of GHRH responses in altered physiologic states and the possible utility of GHRH testing; and c) to explore the efficacy of GHRH and analogs for the treatment of GH deficiency and excess. (a) We have characterized the effect of sex steroids on GHRH responses and episodic GH secretion. Short-term sex steroid treatment increases GH secretion, SmC levels, and responses to GH provocative tests, but does not alter the response to GHRH. Chronic therapy of hypogonadal men increases the amplitude of GH pulsatile secretion. In a comparison of patterns of GH secretion in young and old men and women, the strongest predictor of spontaneous GH secretion is plasma levels of estradiol. (b) We have determined the patterns of response to GHRH during normal aging and during pubertal development. Responses are similar at all these life stages, permitting adult normative data to be used as standards for pediatric studies. Most children with GH deficiency (GHD) respond to GHRH injections with an increase in GH, suggesting that GH deficiency is largely a hypothalamic GHRH deficiency. Among non-responding GHD patients, the majority convert to responders with repeated GHRH priming, indicating that their initial lack of responsivity is due to atrophy of unstimulated somatotrophs rather than to intrinsically abnormal pituitaries. Thus GHRH could be used as therapy for the majority of GHD patients. (c) After demonstration of the efficacy of GHRH for acceleration of growth in a short-term study, we have begun two chronic therapy trials to determine the dose and pattern of GHRH needed for therapy. It appears that GHRH need not be given by frequent pulsatile injections and can restore normal growth rates even when given as a single daily injection. Characterization of even longer-acting GHRH preparations and of ways to enhance the response to a given GHRH dose is now in progress.																										

## Cooperating Units:

Peter Avgerinos, M.D.	BPB, NIMH
Marc Blackman, M.D.	GRC, NIA
William Gahl, M.D.	HGB, NICHD
Philip Gold, M.D.	BPB, NIMH
S. Mitchell Harman, M.D., Ph.D.	GRC, NIA
Eugenia Pavlov, M.D.	GRC, NIA
Rosario D'Agata, M.D.	University of Catania, Italy
Santiago Muzzo, M.D.	INTA, Chile
Ora Pescovitz, M.D.	Dept. of Pediatrics, Univ. of Minnesota
Roger Rittmaster, M.D.	Dept. of Medicine, Dalhousie University
Yi-Fan Shi, M.D.	Chinese Academy of Medical Sciences, Beijing
Sylwester Sobieszczyc, M.D.	University of Poznan/Ludwig Maximilians Universitat
Michael O. Thorner, M.D.	University of Virginia

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00623-03 DEB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Adrenal Physiology and Pathophysiology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. B. Cutler, Jr.

Head

DEB, NICHD

Others: (see attached list)

## COOPERATING UNITS (if any)

(see attached list)

## LAB/BRANCH

Developmental Endocrinology Branch

## SECTION

Section on Developmental Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.2

## PROFESSIONAL

4.0

## OTHER

0.2

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing's syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.



## Other professional personnel

Others:	D. L. Loriaux	Chief	DEB, NICHD
	B. Albertson	Staff Fellow	DEB, NICHD
	P. Avgerinos	Guest Researcher	BPD, NIMH
	K.M. Barnes	Chemist (Tech)	DEB, NICHD
	F. Cassorla	Visiting Scientist	DEB, NICHD
	C. Chik	Guest Researcher	DEB, NICHD
	G. Chrousos	Visiting Scientist	DEB, NICHD
	P. Feuillan	Medical Staff Fellow	DEB, NICHD
	L. Laue	Med. Staff Fellow	DEB, NICHD
	T. Loughlin	Guest Researcher	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	J. Levine Ross	Guest Researcher	DEB, NICHD
	R. Whitcomb	Medical Staff Fellow	DEB, NICHD

## Collaborating Investigators

Chief, Radiology, Clinical Center, NIH (J. Doppman); Chief, SNE, BPB, National Institute of Mental Health (P. Gold); New Mexico State University, Holloman AFB, New Mexico (W. C. Hobson); Senior Investigator, SNB, NINCDS, NIH (E. Oldfield); Staff Radiologist, Radiology, CC, NIH (N. Petronas); Department of Internal Medicine, McMaster University Medical Center (J. Booth); Department of Internal Medicine, University of Dalhousie (R. Rittmaster); Department of Obstetrics and Gynecology, University of Buffalo (A. Munabi); Department of Pediatrics, University of Minnesota (O. Pescovitz).



# ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH

- Z01 HD 00022-13     Renin-Angiotensin System and Aldosterone Regulation  
Greti Aguilera, M.D.
- Z01 HD 00035-14     The Structure and Function of Biologically Active  
Molecules  
Hao-Chia Chen, Ph.D.
- Z01 HD 00146-11     Structure and Function of Chorionic Gonadotropins  
Hao-Chia Chen, Ph.D.
- Z01 HD 00147-11     Mechanism of Action of Peptide Hormones in  
Steroidogenic Cells  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00149-11     Bioassay of Serum Luteinizing Hormone (LH) and  
Chorionic Gonadotropin  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00150-11     Characterization and Purification of LH/hCG Receptors  
and Adenylate Cyclase  
Maria L. Dufau, M.D., Ph.D.
- Z01 HD 00151-11     Receptor-mediated Regulation of Gonadal Function  
Kevin J. Catt, M.D., Ph.D.
- Z01 HD 00184-08     Regulation of Pituitary Hormone Secretion  
Kevin J. Catt, M.D., Ph.D.
- Z01 HD 00187-07     Hormonal Regulation of Cellular Metabolism  
Kuo Ping Huang, Ph.D.
- Z01 HD 00190-04     Adrenocortical Zonation: Regulation of Steroidogenesis  
and Cholesterol Metabolism  
Charles A. Strott, M.D.
- Z01 HD 00191-02     Mechanisms of Neuroendocrine Regulation  
Greti Aguilera, M.D.
- Z01 HD 00192-01     Purification, Immunology and Functional Activity  
of Adrenocortical Proteins  
Charles A. Strott, M.D.
- Z01 HD 00193-01     Angiotensin II Receptors and Activation Mechanisms  
Kevin J. Catt, M.D., Ph.D.





NICHD ANNUAL REPORT  
October 1, 1985 to September 30, 1986

Endocrinology and Reproduction Research Branch

The research programs of the Endocrinology and Reproduction Research Branch are directed at the elucidation of cellular mechanisms involved in hormone secretion and action, and at the investigation of normal and disordered function of the hypothalamic-pituitary system and its effects upon gonadal and adrenal function. These programs include studies on the characterization of peptide hormones and their cellular receptors; the structure-function relationships of peptide and glycoprotein hormones; the regulation of hormone biosynthesis and secretion; and the mechanisms of peptide hormone action in endocrine target cells. Of particular interest are the analysis of pituitary-gonadal and pituitary-adrenal regulation, the control of ovarian activity during the reproductive cycle and pregnancy, and the participation of hormone receptors in the regulation of pituitary, gonadal, and adrenal function. In the current year, research has been extended in several areas of hormone secretion and action, and on the receptor-mediated processes that are responsible for the control of steroid production in endocrine target cells. The role of hormones in cellular regulation has also been examined in selected areas of normal and disordered human endocrine function, and in appropriate animal model systems for the analysis of peptide secretion and the stimulatory and inhibitory control of target-cell function. The staff of the ERRB share common interests in the secretion and mechanisms of action of peptide and glycoprotein hormones, the role of neuropeptides in hypothalamic-pituitary regulation, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system and aldosterone secretion, and the role of phosphorylation in metabolic regulation. The major research programs of the Branch are supervised by the respective senior investigators under the following organizational units within the ERRB.

(a). The Section on Hormonal Regulation (Dr. Kevin Catt) performs research on the control of endocrine target cells by peptide hormones, in particular the characterization, regulation, and activation mechanisms of membrane receptors for gonadotropins, angiotensin II, gonadotropin-releasing hormone (GnRH), and corticotropin-releasing factor (CRF). The receptor-mediated actions of gonadotropin-releasing hormone (GnRH) and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells.

In the pituitary gland, GnRH receptors exhibit prominent variations in number during the ovarian cycle and after changes in steroid feedback, and are modulated by the rate of GnRH secretion from the hypothalamus. In cultured pituitary cells, GnRH receptors undergo down-regulation during exposure to GnRH agonists, followed by a subsequent elevation of sites that is dependent on protein synthesis. GnRH antagonists do not cause receptor down-regulation, but high affinity antagonist analogs bind for extended periods to cause receptor occlusion and prolonged inhibition of GnRH action. Analysis of the rat pituitary GnRH receptor by photoaffinity labeling has revealed two binding subunits of Mr 53,000 and 42,000. The receptor-activated processes leading to gonadotropin secretion are highly calcium dependent, and are initiated by rapid phospholipid hydrolysis with production of arachidonic acid metabolites, diacylglycerol, and inositol phosphates. The role of protein kinase C in gonadotropin secretion is indicated by the ability of phorbol esters and synthetic diacylglycerols to stimulate LH release, and by the redistribution of protein kinase C between cytosol and membrane fractions in GnRH-stimulated gonadotrophs.

It is likely that the effects of arachidonate metabolites are integrated with those of calcium-calmodulin and calcium, phospholipid-dependent protein kinases during the immediate and sustained phases of GnRH-induced gonadotrophin secretion. Studies with dihydropyridine calcium channel agonist and antagonist derivatives, and measurements of cytoplasmic calcium in Quin-2-loaded cells, revealed that increases in intracellular calcium via voltage-sensitive calcium channels partially reproduce GnRH action, and suggest that GnRH causes activation of such channels in the gonadotroph. The increase in cytoplasmic calcium during GnRH action also originates in part from mobilization of internal calcium stores, and its relatively small magnitude is consistent with the concomitant activation of protein kinase C as an intermediate step in GnRH action.

The hormonal control of gonadal endocrine function by gonadotropins and growth factors was studied in ovarian and testicular target cells. The mechanisms of hormonal control of granulosa-cell maturation, and the production of specific receptors and other proteins that mediate hormone action in cells of the reproductive system, were analyzed in cultured granulosa cells. Studies with estrogen antagonists showed that the cAMP-mediated production of LH receptors in FSH-stimulated granulosa cells, demonstrated in earlier studies, was dependent upon the continued actions of estrogen throughout the maturation process. In addition, the ability of aromatase inhibitors to prevent LH receptor expression revealed that sustained production of estrogen by the maturing target cells was essential for FSH-induced granulosa cell differentiation. The inhibitory action of GnRH on ovarian function was found to be reproduced by activators of protein kinase C, indicating that this enzyme has a major role in GnRH action in the ovary as well as in the pituitary gland. In immature granulosa cells, phorbol esters suppressed FSH-induced aromatase activity and cellular aggregation and caused translocation of protein kinase C from cytosol to other cellular compartments. Synthetic diacylglycerols also prevented FSH-induced LH receptor expression and progesterone production, but had less inhibitory effect than phorbol esters on cell aggregation and aromatase activity, and did not cause redistribution of protein kinase C. The prominent suppression of granulosa cell differentiation by phorbol esters reflects their rapid and prolonged action on protein kinase C. In contrast to such inhibitory effects in the immature granulosa cell, activation of protein kinase C in more differentiated cells caused stimulation of cyclic AMP formation and progesterone synthesis. Thus, in the mature granulosa cell the calcium, phospholipid-dependent enzyme is also involved in the regulation of steroidogenesis by hormonal ligands including gonadotropins and GnRH.

The properties of angiotensin II (AII) receptors were studied in the adrenal zona glomerulosa, and the mechanisms leading to stimulation of steroidogenesis were analyzed in isolated glomerulosa cells from the rat and bovine adrenal cortex. AII receptors were further characterized by photoaffinity labeling with a C-terminal azido AII derivative, which possessed high labeling efficiency and was applied to the analysis of receptors in several target tissues. Isolation of the photolabeled AII receptor of the bovine adrenal gland was pursued by detergent solubilization and fractionation by ion exchange, lectin-affinity, and immunoaffinity chromatography. Studies on the actions of AII revealed that the calcium channel agonist, BAY K 8644, increased basal aldosterone production and enhanced the responses to AII and  $K^+$  in a differential manner, by increasing the maximum aldosterone response to AII but not to  $K^+$ . These findings suggest that voltage-sensitive calcium channels are partially operative under basal conditions and are further activated by AII and  $K^+$ . Elevation of cytoplasmic



calcium by AII also depends upon mobilization of intracellular calcium stores by the products of ligand-stimulated phosphoinositide turnover. Microsomal receptors for the putative mediator of calcium mobilization, inositol-1,4,5-trisphosphate ( $IP_3$ ) were identified in adrenal microsomes by binding studies with [ $^{32}P$ ]  $IP_3$ , and show high specificity and affinity ( $K_d$  5 nM) as well as low capacity for  $IP_3$ . The generation of inositol-1,4,5-trisphosphate from phosphatidylinositol biphosphate during AII action was extremely rapid and was accompanied by major production of  $IP_2$  and inositol-4-monophosphate as well as formation of the inactive  $IP_3$  isomer, inositol-1,3,4-trisphosphate. The finding that  $IP_3$  is rapidly degraded to Ins-4-P contrasts with the previous view that Ins-1-P is the major metabolic product, and indicates that Ins-4-P serves as a marker of polyphosphoinositide turnover. The  $IP_3$ -receptor system and the activation of voltage sensitive calcium channels are the major mechanisms involved in the regulation of intracellular calcium by AII and potassium, respectively, in the adrenal zona glomerulosa cell.

The Unit on Endocrine Physiology (Dr. Greti Aguilera) investigates the physiological and pathological aspects of the renin-angiotensin system, with emphasis in the role of AII in the regulation of aldosterone secretion and circulatory homeostasis. AII mediates the increases in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa cell to AII. In addition to the modulatory action of somatostatin and dopaminergic mechanisms as possible regulators of the adrenal sensitivity to AII, atrial natriuretic factor (ANF) is a potent inhibitor of aldosterone secretion. As well as exerting a preferential inhibitory effect on AII-stimulated aldosterone secretion *in vitro*, ANF was shown to antagonize the *in vivo* response of plasma aldosterone to AII infusion and sodium restriction, while ACTH-dependent aldosterone stimulation was unaffected. These actions of ANF provide further support for a role of the peptide in the regulation of adrenal responsiveness to AII. Studies on the mechanism of action of AII revealed a calcium/calmodulin protein kinase that phosphorylates a 100 kDa cytosolic protein in the adrenal glomerulosa zone. This enzyme is different from myosin light chain kinase and its activity was found to be regulated by AII. Previous studies demonstrating the presence of AII receptors in the rat brain were extended to the primate. In monkey brain, AII receptors were found in the circumventricular organs and other limbic structures, all related to the regulation of water intake, blood pressure and autonomic function. In the rat pituitary gland, AII stimulates prolactin secretion after binding to specific receptors located in the lactotrophs. In contrast to the adrenal and vascular AII receptors, pituitary receptors were unaffected by changes in sodium diet and plasma AII levels, but were regulated by estrogens. Incubation of pituitary cells with estradiol increased prolactin responses to AII but caused a time and dose dependent decrease in AII receptors, indicating that the major regulatory effects of estrogens are exerted at post-receptor sites in the pituitary lactotroph.

Studies on the mechanisms of neuroendocrine regulation have focused on the actions of corticotropin releasing factor (CRF) receptors, and the interactions of CRF with other ACTH regulators including vasopressin (VP), angiotensin II (AII), norepinephrine and glucocorticoids. Previous studies revealed that the increases in plasma ACTH after adrenalectomy are accompanied by pituitary CRF receptor down-regulation and desensitization. Further research in rats receiving CRF infusion has demonstrated that sustained exposure of the pituitary to CRF causes CRF receptor loss and a specific decrease in CRF-stimulated

adenylate cyclase activity, which could partially account for the changes following adrenalectomy.

CRF receptors were previously demonstrated in the rat brain. Current studies in the monkey have shown the presence of CRF receptors in the cerebral cortex, limbic system, and related areas of the primate brain. In the peripheral nervous system, the importance of CRF receptors in the adrenal medulla was emphasized by studies in isolated bovine chromaffin cells which demonstrated the ability of CRF to stimulate catecholamine and met-enkephalin secretion. The interactions between ACTH regulators and their mechanism of action were analyzed in rat pituitary cells. In addition to the cyclic AMP-dependent mechanisms by which CRF stimulates the corticotroph, activators of protein kinase C such as phorbol esters, synthetic diacylglycerol and phospholipase C were found to stimulate ACTH secretion. This effect was additive to the stimulatory effect of CRF, but not to those of VP, ALL and norepinephrine, suggesting the involvement of protein kinase C in the action of cyclic AMP-independent stimuli. In studies on glucocorticoid feedback, experiments in isolated pituitary cells demonstrated that the biphasic inhibitory pattern of ACTH secretion observed in vivo also occurs in vitro in the corticotroph. The two inhibitory components have different kinetics and sensitivity to corticosterone and probably involve different mechanisms of action of glucocorticoids in the corticotroph.

(b). The Section on Molecular Endocrinology (Dr. Maria Dufau) investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. A major aspect of this program is concerned with the characterization of gonadal gonadotropin and prolactin receptors, and of the physical and functional relationships of the LH receptor site and adenylate cyclase. Microgram quantities of active lactogen receptors were purified from rat ovaries and subjected to structural and binding studies. The receptor is composed of two dissimilar subunits of Mr 88,000 and 40,000, the latter being probably an integral part of the larger form. Aggregation of receptor subunits and/or holoreceptor was suggested by FLPC fractionation of the free receptor in the presence of non-ionic detergents. Free receptors showed binding activity of Mrs 150,000 and 250,000, and aggregates dissociated upon SDS/mercaptoethanol treatment into the lower molecular forms. These could represent dimeric and trimeric forms of the holoreceptor (80,000). A method for iodination of purified prolactin receptor with preservation of binding activity was used to corroborate the Mr's of the free receptor and hormone-receptor complexes after crosslinking the <sup>125</sup>I-receptor with unlabeled hormone. The results confirmed values initially obtained by crosslinking experiments using labeled hormone and unlabeled receptor. When radiolabeled receptor was used for studies on receptor subunit aggregation, dimeric and trimeric forms were observed. These findings indicate that the detergent soluble receptor appears to contain aggregated forms of holoreceptors. The potential for aggregation of subunits and/or holoreceptors is suggested by these findings, and the mechanism by which aggregation or clustering of prolactin receptors would trigger the biological response remains to be elucidated. Chromatofocusing of free receptors showed three isoforms of pI 4.0, 5.0 and 5.3, indicating glycoprotein and/or phosphorylation heterogeneity. The LH/hCG receptor was also purified, by sequential wheat germ lectin-Sepharose and hCG Sepharose chromatography, which also allows purification of lactogen receptor from the initial starting material. The LH receptor was identified as a single protein of Mr 70,000. This technique is simple and



allows purification of microgram amounts of active receptor suitable for structural studies, microsequencing, and functional reconstitution studies.

The receptor-mediated actions of gonadotropins and other hormones were further analyzed in the testicular Leydig cell. The inability of the fetal/immature Leydig cell to be desensitized by gonadotropin, a characteristic of the adult cell, is due to the absence of an estradiol-mediated regulation of the androgen pathway (17 $\alpha$ -hydroxylase/17/20 demolase). In fetal rat Leydig cell cultures employed to analyze this differential response, estradiol caused an up-regulation of its receptor and an induction of the regulatory mechanism of the androgen pathway. The absence of this endogenous regulation in fetal life is due to very low levels of aromatase activity, undetectable estradiol production, and consequent low levels of estradiol receptor. The above explains the low levels of estradiol regulated 27K protein that is present in fetal cells and increased by E<sub>2</sub> treatment. This long-lived protein is undetectable post-natally and increases with age, and is present in cytoplasmic matrix near the cisternae of RER and SER. Future research with this system will help to clarify mechanisms responsible for emergence of the adult cell population. These cultures were also used for studies on GnRH agonists, which cause a lesion of microsomal enzymes that markedly inhibits steroid production. GnRH receptors were undetectable in fetal testes, appeared post-natally and increased with age. In fetal cultures, GnRH receptors were up-regulated by GnRH and reduced by LH treatment. Thus, GnRH-related peptides acting via GnRH receptors can influence the actions of LH on the fetal Leydig cell population. Opiate receptors and actions were found to be present in Sertoli cells. Opiate up-regulated its receptor and reduced FSH-stimulated androgen binding protein. Opioids of Leydig cell origin may modulate Sertoli cell function and influence the transport of testosterone to germinal cells. Testicular converting enzyme in mature rats was mainly associated with germinal cells (spermatids), results consistent with the effects of hormonal treatment. In contrast, the Leydig cells contain low quantities of converting enzyme, about 5% of the amount present in spermatids. Thus, locally produced angiotensin II could modulate the control of Leydig cell secretion and tubule function by gonadotropins.

The bioassay of serum LH and hCG was applied to further studies on the secretion of bioactive LH. The bioactivity of circulating LH appears to be rapidly modulated by gonadal steroids (i.e. normal men have higher B:I than cycling females; castration in rats decrease the B:I). Also, the decrease in B:I of LH in castrated animals and differences in B:I observed in men vs cycling females could be related to increased LH secretion and/or production rate. Previous estimates of LH blood production derived from immunoassay data markedly underestimated the quantity of bioactive LH secreted in man. The estimated bioactive blood production rate is about 30 fold higher than for LH immunoactivity, values that are in accord with the plasma B:I ratio of 4 in normal men. The metabolic clearance rate for LH bioactivity was 33% less than that for immunoactivity. These results indicate that high endogenous production rates represent a predominant factor responsible for the high B:I ratio characteristic of plasma LH in normal men. In the female, as previously observed with testosterone pulsation in men it is unlikely that a temporal relationship exists between individual bioactive LH and pulses of progesterone secreted by the late corpus luteum. In both sexes, opioids slow GnRH pulses at the hypothalamic level and cause inhibition of LH secretion. The opiate antagonist, naltrexone, increases pulse frequency and mean integrated bioactive LH. Dissection of an early releasable LH pool by a single dose of IV naloxone could be of value to

complement the GnRH test in physiopathological exploration of hypothalamic function in man. Steroids can modify GnRH secretion and estradiol replacement reinstates opioid suppression of bioactive LH secretion in orchidectomized patients with testicular feminization. Also, direct inhibitory effects of opioids on LH secretion, through high affinity pituitary receptors, could further modulate LH bioactivity. Using an in vitro lactogen bioassay, definite prolactin pulsations were detected at all stages of the cycle and B:I ratios were below or near unity. In contrast, serum B:I ratios were elevated during the rat proestrus surge. Prolactin biopotency is much higher in the rat than in the human and increases at proestrous, reflecting the physiological relevance of this hormone to luteal function in this species.

(c). The Section on Adrenal Cell Biology. (Dr. C. Strott) investigates the physiology and regulation of adrenal steroidogenesis, by characterization of cellular steroid binding proteins and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. The guinea pig, a cortisol-producing species, is employed as a model for studies on the regulation of steroidogenesis in the adrenal cortex, and the development and regulation of cellular zonation in the adrenal cortex.

The adrenal cortex of the guinea pig is composed of chromatically distinct outer and inner zones which can be separated by microdissection. In studies exploring the responsivity of the two zones to ACTH, the following observations have been made: 1) there is no steroidogenic response to ACTH by cells isolated from the inner zone; 2) cholesterol side-chain cleavage activity (rate-limiting in steroidogenesis) is significantly lower in the inner zone and is not modulated by ACTH, stress, or chronic dexamethasone suppression; 3) the content of cholesterol is 3-4 times higher in the outer zone. Thus, the guinea pig presents an interesting animal model to investigate steroidogenesis, cholesterol metabolism, and the mechanism of action of ACTH by performing experiments on the two adrenocortical zones in a parallel fashion. The regulation of adrenocortical steroidogenesis by ACTH is complex and only partially understood. The accepted obligatory steps include: stimulation of plasma membrane adenylate cyclase, increase in intracellular cAMP, and activation of cAMP-dependent protein kinase. The role of other kinases such as  $\text{Ca}^{2+}$ /calmodulin- and  $\text{Ca}^{2+}$ /phospholipid-dependent protein kinase is less clear. A large number of proteins (membranous and soluble) which are phosphorylated in response to ACTH have been reported. To date, however, no regulatory phosphoprotein has been identified. Phosphoprotein phosphatases have not been examined. Based on the use of inhibitors of protein and RNA synthesis, a critical role for protein synthesis in the stimulation of adrenal steroidogenesis by ACTH has been proposed. Adrenal steroid production is rapidly activated and deactivated ( $\sim 2$  min); such a process is considered too rapid to involve regulation at the level of translation. Regulation would, however, be compatible with protein modification. It is now well established that the covalent modification of protein is a crucial mechanism by which cellular processes are regulated. It is essential to identify and characterize the modified (eg. phosphorylated or dephosphorylated) steroidogenic regulatory protein. Such is a goal of the comparative approach using the guinea pig adrenal cortex model.

The role of non-catalytic proteins in the adrenocortical steroidogenic process is vague and speculative. Non-catalytic proteins have been implicated in a variety of ways such as regulation of cholesterol side-chain cleavage activity



(the rate-limiting step in steroidogenesis), cholesterol and pregnenolone transport mechanisms, secretory processes, etc. However, to date, no non-catalytic protein has been completely isolated and characterized for the adrenal cortex. The elusive steroidogenic regulatory protein remains to be identified. The adrenal cortex of the guinea pig contains specific steroid-binding proteins which have been only partially purified and characterized; their function is as yet undetermined. For instance, there are proteins which specifically bind cholesterol, cholesteryl sulfate, pregnenolone, and pregnenolone sulfate. These proteins are of great interest because the rate-limiting reaction in steroidogenesis is the conversion of cholesterol to pregnenolone or cholesteryl sulfate to pregnenolone sulfate. The pregnenolone-binding protein, which is isolated from the high speed soluble fraction, has been purified to the greatest extent, but the best current preparations still contain numerous contaminants. To further purify the pregnenolone-binding protein, two approaches are currently being used: 1) development of an affinity probe, 2) generation of antibodies to proteins electroeluted from sodium dodecyl sulfate gel slices. Several antisera have now been generated which are presently being examined by Western blot analysis as well as for interaction with the pregnenolone-binding protein using the technique of sucrose density gradient analysis. It is planned in the near future to obtain the capability to utilize high performance liquid chromatography for purifying isolated gel protein bands. In addition to purifying steroid-binding proteins, it is becoming increasingly apparent that selective phosphoproteins will need to be isolated and characterized. The latter task will be difficult and time-consuming, but will be necessary if a specific functional significance is to be ascertained for a particular phosphorylated (or dephosphorylated) protein.

(d) The Section on Molecular Structure and Protein Chemistry. (Dr. H.C. Chen) conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides and proteins. This includes the identification and synthesis of unusual structure and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis.

A peptide sequence in proteins, -Pro-Lys-Lys-Lys-Arg-Lys-Val-, known to lead to translocation into the nucleus, was synthesized as a N-trifluoroacetylated sulfohydroxysuccinimido ester. After coupling to avidin and removal of trifluoroacetyl groups by piperidine, a conjugate that has two peptide units linked through the COOH-terminal group of the peptide to two  $\epsilon$ -NH<sub>2</sub>-groups in the tetrameric avidin molecule was obtained. This conjugate exhibited full biotin-binding activity. Similarly, a peptide of 22 amino acid residues, designated as Differentiation Peptide B, was synthesized by a solid phase method and derivatized as N<sup>α</sup>N<sup>ε</sup>-trifluoroacetyl pentafluorophenyl ester, and then conjugated to bovine thyroglobulin. In both cases, defined linkages between peptide and protein were achieved. Further research was performed on the biological properties of dimeric gonadotropin releasing hormone agonists and antagonists. Three dimeric des-Gly<sup>10</sup>-[D-Lys<sup>6</sup>]-GnRH-NHEt cross-linked at  $\epsilon$ -amino group with -Gly<sup>n</sup>-COCH<sub>2</sub>-CO-Gly<sup>n</sup>- (n=0, 1, 2) exhibited increased biological activities and mast cell histamine releasing activity over the monomer. However, a dimer (n=1) was found to display a substantial increase of a post-coital antifertility effect over the increase of histamine releasing side-effect. A similar approach was taken in the synthesis and purification of dimers of a GnRH antagonist of the following sequence: Ac-D-pClPhe-D-pCLPhe-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>. Antisera against the agonist and the antagonist monomers

were also generated. In other studies determinations of amino acid compositions and partial amino acid sequence of induced NAD(P)H:menadione oxidoreductase allowed deduction of correct reading frame of the cloned cDNA.

The role of carbohydrate structures of human chorionic gonadotropin (hCG) in gonadotropic action and subunit association was the focus of this study. Production and purification of hCG specific antibodies were also undertaken.

The measurement of the rate of fluorescence enhancement of 1-anilinonaphthyl-8-sulfonate when complexed with intact or HF-deglycosylated hCG revealed that the subunits of deglycosylated hCG reassociated nearly 10-fold faster than unmodified subunits. Studies on the reassociation rate of hybrid subunits from the intact  $\beta$ -subunit of ovine luteinizing hormone and subunits of intact and modified hCG suggest that carbohydrate moieties in the  $\alpha$ -subunit dominate the reassociation behavior. Antibodies raised against HF-deglycosylated hCG and a bivalent  $F(ab')_2$  fragment prepared and purified from a sequence-specific anti-carboxymethylated hCG $\beta$  antibody were shown to enhance stimulation of cAMP and progesterone production in rat differentiated granulosa cells preincubated with the modified hCG. These findings are consistent with the notion that hormonal activation may be caused by cross-linking and/or microaggregation of the HF-hCG:receptor complex. However, antibodies which bind to an appendage of HF-hCG too flexible and distant from the complex or which have the ability to strip off hormone from the receptor complex were ineffective in eliciting activation.

(e). The Section on Metabolic Regulation (Dr. K.-P. Huang) studies the regulation and hormonal control of glycogen metabolism in normal and diabetic tissues, and the activities of glycogen synthase and phosphorylase kinase.

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C, a  $Ca^{2+}$ /phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those by many hormones and growth factors. Three isozymic forms of rat brain protein kinase C have been purified to near homogeneity. Polyclonal and monoclonal antibodies against these enzymes were prepared for the immunochemical characterization. These isozymes are distinguishable by peptide mapping and immunological analysis, indicating that they are products of different genes. The various protein kinase C isozymes were found to be enriched in different regions of rat brain and expressed differentially during brain development. Activation of protein kinase C in cells was studied by using EL4 thymoma cells which can be induced to secrete interleukin-2 in response to tumor-promoting phorbol esters. These compounds promote the translocation of protein kinase C from cytosol to the particulate fraction and a rapid degradation of the enzyme. The phorbol ester-induced translocation and degradation of protein kinase C may be early events in the secretion of interleukin-2. In vitro, tryptic degradation of purified protein kinase C generates active forms of the kinase and phorbol ester-binding protein that are active in the absence of  $Ca^{2+}$ . The structure-function relationships of protein kinase C were investigated by using monoclonal antibody specific for the



phorbol ester-binding domain. This antibody reduces the binding of phorbol ester to the enzyme without inhibiting the kinase activity. Protein kinase C activity was shown to be regulated by autophosphorylation, and the resulting phosphorylated kinase exhibited higher affinity for  $\text{Ca}^{2+}$  and phorbol esters.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00022-13 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Renin-Angiotensin System and Aldosterone Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: G. Aguilera Research Biologist ERRB, NICHD

Others: K.J. Catt Head ERRB, NICHD  
M.A. Millan Sr. Staff Fellow ERRB, NICHD  
T. Kigoshi Visiting Fellow ERRB, NICHD  
W.P. Hausdorff Guest Researcher ERRB, NICHD

## COOPERATING UNITS (if any)

DEB, NICHD (M.P. Platia)

Lab Clin. Sci., NIMH (D. Jacobowitz)

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation (Endocrine Physiology Unit)

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD. 20205

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of this project is to analyze physiological and pathological aspects of the renin-angiotensin system, with emphasis in the role of AII in the regulation of aldosterone secretion and circulatory homeostasis. AII mediates the increases in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa cell to AII. In addition to the modulatory action of somatostatin and dopaminergic mechanisms as possible regulators of the adrenal sensitivity to AII, atrial natriuretic factor (ANF) is a potent inhibitor of aldosterone secretion. As well as exerting a preferential inhibitory effect on AII-stimulated aldosterone secretion in vitro, experiments in vivo showed that ANF antagonized the increases in plasma aldosterone following AII infusion and sodium restriction, while ACTH-dependent aldosterone stimulation was unaffected. These actions of ANF are in support of a role for the peptide in the regulation of adrenal responsiveness to AII. Studies on the mechanism of action of AII revealed a novel calcium/calmodulin protein kinase that phosphorylates a 100 kDa cytosolic protein in the adrenal glomerulosa zone. This enzyme is different from myosin light chain kinase and its activity was shown to be regulated by AII. Previous studies demonstrating the presence of AII receptors in the rat brain were extended to the primate. In monkey brain, AII receptors were found in the circumventricular organs and other limbic structures, all related to the regulation of water intake, blood pressure and autonomic function. In the rat pituitary gland, AII stimulates prolactin secretion after binding to specific receptors located in the lactotrophs. In contrast to the adrenal and vascular AII receptors, pituitary receptors were unaffected by changes in sodium diet and plasma AII levels, but were regulated by estrogens. Incubation of pituitary cells with estradiol increased prolactin responses to AII but caused a time and dose dependent decrease in AII receptors, indicating that the major regulatory effects of estrogens are exerted at post-receptor sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00035-14 ERRB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Structure and Function of Biologically Active Molecules		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	H. C. Chen	Head ERRB, NICHD
Others:	K. J. Catt	Chief ERRB, NICHD
	J. L. Morell	Research Chemist ERRB, NICHD
	J. H. Brown	Research Chemist ERRB, NICHD
	Y. Kitajima	Visiting Fellow ERRB, NICHD
COOPERATING UNITS (if any)  Laboratory of Microbiology and Immunity, NIDR, NIH (W.A. Hook) Laboratory of Developmental Pharmacology, NICHD (D.W. Nebert)		
LAB/BRANCH Endocrinology and Reproduction Research Branch		
SECTION Section on Molecular Structure & Protein Chemistry		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This project focuses on structural design, chemical synthesis, and modification of molecules important to reproductive and developmental biology.</p> <p>A. A peptide sequence in proteins, -Pro-Lys-Lys-Lys-Arg-Lys-Val-, known to lead to translocation into the nucleus, was synthesized as a N-trifluoroacetylated sulfohydroxysuccinimido ester. After coupling to avidin and removal of trifluoroacetyl groups by piperidine, a conjugate that has two peptide units linked through the COOH-terminal group of the peptide to two <math>\epsilon</math>-NH<sub>2</sub>-groups in the tetrameric avidin molecule was obtained. This conjugate exhibited full biotin-binding activity. Similarly, a peptide of 22 amino acid residues, designated as Differentiation Peptide B, was synthesized by a solid phase method and derivatized as N<sup>α</sup>N<sup>ε</sup>-trifluoroacetyl pentafluorophenyl ester, and then conjugated to bovine thyroglobulin. In both cases, defined linkages between peptide and protein were achieved.</p> <p>B. Dimeric gonadotropin releasing hormone agonists and antagonists. Three dimeric des-Gly<sup>10</sup>-[D-Lys<sup>6</sup>]-GnRH-NH<sub>2</sub> cross-linked at <math>\epsilon</math>-amino group with -Gly-COCH<sub>2</sub>-CO-Gly<sub>n</sub>- (n=0, 1, 2) exhibited increased biological activities and mast cell histamine releasing activity over the monomer. However, a dimer (n=1) was found to display a substantial increase of a post-coital antifertility effect over the increase of histamine releasing side-effect. A similar approach was taken in the synthesis and purification of dimers of a GnRH antagonist of the following sequence: Ac-D-pClPhe-D-pCLPhe-D-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-D-Ala-NH<sub>2</sub>. Antisera against the agonist and the antagonist monomers were also generated.</p> <p>C. Determinations of amino acid compositions and partial amino acid sequence of induced NAD(P)H:menadione oxidoreductase allowed deduction of correct reading frame of the cloned cDNA.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00146-11 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Chorionic Gonadotropin

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. C. Chen	Head	ERRB, NICHD
Others:	K. J. Catt	Chief	ERRB, NICHD
	P. Feng	Visiting Fellow	ERRB, NICHD
	J. H. Brown	Research Chemist	ERRB, NICHD

## COOPERATING UNITS (if any)

Department of Chemistry, Georgetown University (D. C. H. Yang)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Structure &amp; Protein Chemistry

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unraduced type. Do not exceed the space provided.)

The role of carbohydrate structures of human chorionic gonadotropin (hCG) in gonadotropic action and subunit association was the focus of this study. Production and purification of hCG specific antibodies were also undertaken.

A. The measurement of the rate of fluorescence enhancement of 1-anilidonaphthyl-8-sulfonate when complexed with intact or HF-deglycosylated hCG revealed that the subunits of deglycosylated hCG reassociated nearly 10-fold faster than unmodified subunits. Studies on the reassociation rate of hybrid subunits from the intact  $\beta$ -subunit of ovine luteinizing hormone and subunits of intact and modified hCG suggest that carbohydrate moieties in the  $\alpha$ -subunit dominate the reassociation behavior.

B. Antibodies raised against HF-deglycosylated hCG and a bivalent  $F(ab')_2$  fragment prepared and purified from a sequence-specific anti-carboxymethylated hCG $\beta$  antibody were shown to enhance stimulation of cAMP and progesterone production in rat differentiated granulosa cells preincubated with the modified hCG. These findings are consistent with the notion that hormonal activation may be caused by cross-linking and/or microaggregation of the HF-hCG:receptor complex. However, antibodies which bind to an appendage of HF-hCG too flexible and distant from the complex or which have the ability to strip off hormone from the receptor complex were ineffective in eliciting activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00147-11 ERRB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) <u>Mechanism of Action of Peptide Hormones in Steroidogenic Cells</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)		
PI:	M.L. Dufau	Head ERRB, NICHD
Others:	C-H. Tsai-Morris	Staff Fellow ERRB, NICHD
	Andrea Fabbri	Guest Researcher ERRB, NICHD
	C.A. Winters	Chemist ERRB, NICHD
	A. Kahnum	Visiting Fellow ERRB, NICHD
	T. Minegishi	Visiting Fellow ERRB, NICHD
COOPERATING UNITS (if any) Contract for preparation of gonadal cells and cell fractions DHEW-275-82-2823		
LAB/BRANCH Endocrinology and Reproduction Research Branch		
SECTION Section on Molecular Endocrinology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	3.55	PROFESSIONAL 3.05 OTHER 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided ) The goal of this project is to understand the steps involved in the control of testicular function. The inability of the fetal/immature Leydig cell to be desensitized by gonadotropin, a characteristic of the adult cell, is due to the absence of an estradiol(E <sub>2</sub> )-mediated regulation of the androgen pathway (17α-hydroxylase/17/20 demolase). In fetal rat Leydig cell cultures employed to analyze this differential response, E <sub>2</sub> caused an up-regulation of its receptor and an induction of the regulatory mechanism of the androgen pathway. The absence of this endogenous regulation in fetal life is due to very low levels of aromatase activity, undetectable E <sub>2</sub> production, and consequent low levels of E <sub>2</sub> receptor. The above explains the low levels of E <sub>2</sub> -regulated 27K protein that is present in fetal cells and increased by E <sub>2</sub> treatment. This long-lived protein is undetectable post-natally and increases with age, and is present in cytoplasmic matrix near the cisternae of RER and SER. Future research with this system will help to clarify mechanisms responsible for emergence of the adult cell population. These cultures were also used for studies on GnRH agonists, which cause a lesion of microsomal enzymes that markedly inhibits steroid production. GnRH receptors were undetectable in fetal testes, appeared post-natally and increased with age. In fetal cultures, GnRH receptors were up-regulated by GnRH and reduced by LH treatment. Thus, GnRH receptors via GnRH related peptides can influence the actions of LH on the fetal cell population. Opiate receptors and actions were present in Sertoli cells. Opiate up-regulated its receptor and reduced FSH-stimulated androgen binding protein. Opioids of Leydig cell origin may modulate Sertoli cell function and influence the transport of testosterone to germinal cells. Testicular converting enzyme in mature rats was mainly associated with germinal cells (spermatids), results consistent with hormonal studies. The Leydig cells contain low quantities of converting enzyme, 0.46 mIU/ng vs 29.1 mIU/mg in spermatids. Thus, tubular and locally produced angiotensin II could negatively modulate LH stimulation of Leydig cells and also modulate tubule function.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00149-11 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.L. Dufau Head ERRB, NICHD

Others: K.J. Catt Chief ERRB, NICHD

M. Blank Guest Scientist ERRB, NICHD

M. Ching Guest Scientist ERRB, NICHD

COOPERATING UNITS (if any) Dept. Medicine, Charlottesville, VA, Dept. of Pediatrics, the GS Mott Children's Hospital, Univ. Michigan, Ann Arbor, MI, Contract for prep. of gonadal cells and cell fractions DHEW 275-82-2823, V Clinica Medica, Univ. Rome, "La Sapienza", Rome, Italy.

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Molecular Endocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.45

## PROFESSIONAL:

0.45

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The bioactivity of circulating LH appears to be rapidly modulated by gonadal steroids (i.e. normal men have higher B:I than cycling females; castration in rats decrease the B:I). Also, the decrease in B:I of LH in castrated animals and differences in B:I observed in men vs cycling females could be related to increased LH secretion and/or production rate. Previous estimates of LH blood production derived from immunoassay data markedly underestimated the quantity of bioactive LH secreted in man. The estimated bioactive blood production rate is about 30 fold higher than for LH immunoactivity, values that are in accord with the plasma B:I ratio of 4 in normal men. Metabolic clearance rate for LH bioactivity was 33% less than that for immunoactivity. Results indicate that high endogenous production rates represent a predominant factor responsible for the high B:I ratio characteristic of plasma LH in normal men. As previously observed with testosterone pulsation in men it is unlikely that a temporal relationship exists between individual bioactive LH and pulses of progesterone secreted by the late corpus luteum. Opioids slow GnRH pulses at the hypothalamic level and cause inhibition of LH secretion. The opiate antagonist, naltrexone, increases pulse frequency and mean integrated bioactive LH. Dissection of an early releasable LH pool by a single dose of IV naloxone could be of value to complement the GnRH test in physiopathological exploration of hypothalamic function in man. Steroids can modify GnRH secretion and  $E_2$  replacement reinstates opioid suppression of bioactive LH secretion in orchidectomized patients with testicular feminization. Also, direct inhibitory effects of opioids on LH secretion, through high affinity pituitary receptors, could further modulate LH bioactivity. Using an in vitro lactogen bioassay, definite prolactin pulsations were detected at all stages of the cycle and B:I ratios were below or near unity. In contrast, serum B:I ratios were elevated during the rat proestrus surge. Prolactin biopotency is much higher in the rat than in the human and increases at proestrous, reflecting the physiological relevance of this hormone to luteal function in this species.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00150-11 ERRB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M.L. Dufau	Head ERRB, NICHD
Others:	C. Winters	Chemist ERRB, NICHD
	S. Kusuda	Visiting Associate ERRB, NICHD
	A. Khanum	Visiting Fellow ERRB, NICHD
COOPERATING UNITS (if any) Contract for preparation of gonadal cells and cell fractions DHEW-275-82-2823		
LAB/BRANCH Endocrinology and Reproduction Research Branch		
SECTION Section on Molecular Endocrinology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.50
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p>             This aspect of the program is concerned with characterization of gonadotropin and prolactin receptors of the gonads and of the physical and functional relationships of the LH receptor site and adenylate cyclase. We have devised a procedure for purification of microgram quantities of active lactogen receptors from rat ovaries. The receptor is composed of two dissimilar subunits of Mr 88,000 and 40,000, the latter being probably an integral part of the larger form. Aggregation of receptor subunits and/or holoreceptor has been suggested by FLPC fractionation of the free receptor in the presence of non-ionic detergents. Free receptors showed binding activity of Mrs 150,000 and 250,000, and aggregates dissociated upon SDS/mercaptoethanol treatment into the lower molecular forms. These could represent dimeric and trimeric forms of the holoreceptor (80,000). A method for iodination of purified prolactin receptor with preservation of activity was used to corroborate the Mr's of the free receptor and hormone-receptor complexes after crosslinking the <sup>125</sup>I-receptor with unlabeled hormone. Results confirmed values initially obtained by crosslinking experiments using labeled hormone and unlabeled receptor. When radiolabeled receptor was used for studies on receptor subunit aggregation, dimeric and trimeric forms were observed. Our results indicate that the detergent soluble receptor appeared to be aggregated forms of holoreceptors. The potential for aggregation of subunits and/or holoreceptors is suggested by these findings, and the mechanism by which aggregation or clustering of prolactin receptors would trigger the biological response remains to be elucidated. Chromatofocusing of free receptors showed three isoforms of pI 4.0 5.0 and 5.3 indicating glycoprotein and or phosphorylation heterogeneity. We have purified the LH/hCG receptor on wheat germ lectin-Sepharose and hCG Sepharose, which also allows purification of lactogen receptor from the initial starting material. The LH receptor was identified as a single protein Mr 70,000. The technique is simple and allows purification of microgram amounts of active receptor suitable for structural studies microsequencing, and functional reconstitution.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00151-11 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptor-Mediated Regulation of Gonadal Function

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. J. Catt	Head	ERRB, NICHD
	M. Knecht	Sr. Staff Fellow	ERRB, NICHD
Others:	H.-C. Chen	Head, SMSPC	ERRB, NICHD
	P. Feng	Visiting Fellow	ERRB, NICHD
	M. Zilberstein	Visiting Fellow	ERRB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.0

## PROFESSIONAL:

3.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The hormonal control of gonadal endocrine function by gonadotropins and growth factors is studied in ovarian and testicular target cells. In the ovary, the mechanisms of hormonal control of granulosa-cell maturation, and the production of specific receptors and other proteins that mediate hormone action in cells of the reproductive system, were analyzed in cultured granulosa cells. Studies with estrogen antagonists showed that the cAMP-mediated production of LH receptors in FSH-stimulated granulosa cells, demonstrated in earlier studies, was dependent upon the continued actions of estrogen throughout the maturation process. In addition, the ability of aromatase inhibitors to prevent LH receptor expression revealed that sustained production of estrogen by the maturing target cells was essential for FSH-induced granulosa cell differentiation. The inhibitory action of GnRH on ovarian function was found to be reproduced by activators of protein kinase C, indicating that this enzyme has a major role in GnRH action in the ovary as well as in the pituitary gland. In immature granulosa cells, phorbol esters suppressed FSH-induced aromatase activity and cellular aggregation and caused translocation of protein kinase C from cytosol to other cellular compartments. Synthetic diacylglycerols also prevented FSH-induced LH receptor expression and progesterone production, but had less inhibitory effect than phorbol esters on cell aggregation and aromatase activity, and did not cause redistribution of protein kinase C. The prominent suppression of granulosa cell differentiation by phorbol esters reflects their rapid and prolonged action on protein kinase C. In contrast to such inhibitory effects in the immature granulosa cell, activation of protein kinase C in more differentiated cells caused stimulation of cyclic AMP formation and progesterone synthesis. Thus, in the mature granulosa cell the calcium, phospholipid-dependent enzyme is also involved in the regulation of steroidogenesis by hormonal ligands including gonadotropins and GnRH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00184-08 ERRB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Regulation of Pituitary Hormone Section		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	K. J. Catt	Head, SHR ERRB, NICHD
Others:	M. L. Dufau	Head, MES ERRB, NICHD
	G. Aguilera	Research Biologist ERRB, NICHD
	R. O. Morgan	Staff Fellow ERRB, NICHD
	J. P. Chang	Guest Researcher ERRB, NICHD
	K. Tasaka	Visiting Fellow ERRB, NICHD
COOPERATING UNITS (if any) Dept. of Anatomy, University of Texas Medical Branch, Galveston, Texas (G. Childs); Contract for preparation of adrenal and pituitary cells N01-HD-0-2806		
LAB/BRANCH Endocrinology and Reproduction Research Branch		
SECTION Section on Hormonal Regulation		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The hypothalamic control of reproductive function is expressed through the receptor-mediated actions of GnRH on the pituitary gonadotroph. GnRH receptors in the pituitary gland exhibit prominent variations in number during the ovarian cycle and after changes in steroid feedback, and are modulated by the rate of GnRH secretion from the hypothalamus. In cultured pituitary cells, GnRH receptors undergo down-regulation during exposure to GnRH agonists, followed by a subsequent elevation of sites that is dependent on protein synthesis. GnRH antagonists do not cause receptor down-regulation, but high affinity antagonist analogs bind for extended periods to cause receptor occlusion and prolonged inhibition of GnRH action. Analysis of the rat pituitary GnRH receptor by photoaffinity labeling reveals two binding subunits of Mr 53,000 and 42,000. The receptor-activated processes leading to gonadotropin secretion are highly calcium dependent, and are initiated by rapid phospholipid hydrolysis with production of arachidonic acid metabolites, diacylglycerol, and inositol phosphates. The role of protein kinase C in gonadotrophin secretion is indicated by the ability of phorbol esters and synthetic diacylglycerols to stimulate LH release, and by the redistribution of protein kinase C between cytosol and membrane fractions in GnRH-stimulated gonadotrophs. It is likely that the effects of arachidonate metabolites are integrated with those of calcium-calmodulin and calcium, phospholipid-dependent protein kinases during the immediate and sustained phases of GnRH-induced gonadotrophin secretion. Studies with dihydropyridine calcium channel agonist and antagonist derivatives, and measurements of cytoplasmic calcium in Quin-2-loaded cells, revealed that increases in intracellular calcium via voltage-sensitive calcium channels partially reproduce GnRH action, and suggest that GnRH causes activation of such channels in the gonadotroph. The increase in cytoplasmic calcium during GnRH action also originates in part from mobilization of internal calcium stores, and its relatively small magnitude is consistent with concomitant activation of protein kinase C as an intermediate step in GnRH action.		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00187-07 ERRB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Hormonal Regulation of Cellular Metabolism		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <div>           PI: K.-P. Huang             Others: A. M. Goheer                      F. Huang                      H. Nakabayashi                      Y. Yoshida         </div> <div>           Senior Staff Fellow            Expert            Visiting Fellow            Visiting Fellow         </div> <div>           ERRB, NICHD             ERRB, NICHD            ERRB, NICHD            ERRB, NICHD         </div> </div>		
COOPERATING UNITS (if any) Laboratory of Developmental and Molecular Immunity NICHD, NIH (E. Hanna); Laboratory of Cell Biology, NHLBI, NIH (M. Flavin); Section on Growth Factors, NICHD, NIH (G. Guroff)		
LAB/BRANCH Endocrinology and Reproduction Branch		
SECTION Section on Metabolic Regulation		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda MD 20892		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 4.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C, a $Ca^{2+}$ /phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those by many hormones and growth factors. Three isozymic forms of rat brain protein kinase C have been purified to near homogeneity. Polyclonal and monoclonal antibodies against these enzymes were prepared for the immunochemical characterization. These isozymes are distinguishable by peptide mapping and immunological analysis, indicating that they are products of different genes. The various protein kinase C isozymes were found to be enriched in different regions of rat brain and expressed differentially during brain development. Activation of protein kinase C in cells was studied by using EL4 thymoma cells which can be induced to secrete interleukin-2 in response to tumor-promoting phorbol esters. These compounds promote the translocation of protein kinase C from cytosol to the particulate fraction and a rapid degradation of the enzyme. The phorbol ester-induced translocation and degradation of protein kinase C may be early events in the secretion of interleukin-2. <u>In vitro</u> , tryptic degradation of purified protein kinase C generates active forms of the kinase and phorbol ester-binding protein that are active in the absence of $Ca^{2+}$ . The structure-function relationships of protein kinase C were investigated by using monoclonal antibody specific for the phorbol ester-binding domain. This antibody reduces the binding of phorbol ester to the enzyme without inhibiting the kinase activity. Protein kinase C activity was shown to be regulated by autophosphorylation, and the resulting phosphorylated kinase exhibited higher affinity for $Ca^{2+}$ and phorbol esters.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00190-04 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adrenocortical zonation: regulation of steroidogenesis and cholesterol metabolism

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.A. Strott Head ERRB, NICHD

Others: M. Kubo Visiting Fellow ERRB, NICHD  
C.D. Lyons Bio. Lab. Tech. ERRB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Adrenal Cell Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.25

## PROFESSIONAL:

1.75

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The adrenal cortex of the guinea pig is composed of chromatically distinct outer and inner zones which can be separated by microdissection. In studies exploring the responsivity of the two zones to ACTH, the following observations have been made: 1) there is no steroidogenic response to ACTH by cells isolated from the inner zone; 2) cholesterol side-chain cleavage activity (rate-limiting in steroidogenesis) is significantly lower in the inner zone and is not modulated by ACTH, stress, or chronic dexamethasone suppression; 3) the content of cholesterol is 3-4 times higher in the outer zone. Thus, the guinea pig presents an interesting animal model to investigate steroidogenesis, cholesterol metabolism, and the mechanism of action of ACTH by performing experiments on the two adrenocortical zones in a parallel fashion. The regulation of adrenocortical steroidogenesis by ACTH is complex and only partially understood. The accepted obligatory steps include: stimulation of plasma membrane adenylate cyclase, increase in intracellular cAMP, and activation of cAMP-dependent protein kinase. The role of other kinases such as  $\text{Ca}^{2+}$ /calmodulin- and  $\text{Ca}^{2+}$ /phospholipid-dependent protein kinase is less clear. A large number of proteins (membranous and soluble) which are phosphorylated in response to ACTH have been reported. To date, however, no regulatory phosphoprotein has been identified. Phosphoprotein phosphatases have not been examined. Based on the use of inhibitors of protein and RNA synthesis, a critical role for protein synthesis in the stimulation of adrenal steroidogenesis by ACTH has been proposed. Adrenal steroid production is rapidly activated and deactivated ( $\sim 2$ min); such a process is considered too rapid to involve regulation at the level of translation. Regulation would, however, be compatible with protein modification. It is now well established that the covalent modification of protein is a crucial mechanism by which cellular processes are regulated. It is essential to identify and characterize the modified (eg. phosphorylated or dephosphorylated) steroidogenic regulatory protein. Such is a goal of the comparative approach using the guinea pig adrenal cortex model.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 HD 00191-02 ERRB
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Neuroendocrine Regulation		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
<b>PI:</b>  <b>Others:</b>	G. Aguilera  K.J. Catt A.B. Abou-Samra M.A. Millan	Research Biologist  Head Visiting Fellow Sr. Staff Fellow
ERRB, NICHD  ERRB, NICHD ERRB, NICHD		
<b>COOPERATING UNITS</b> (if any)		
Lab. Clin. Science, NIMH (D. Jacobowitz) Section Exp. Nutrition FDA (J. Harwood) Dep. Psych. U. California (R. Hauger)		
<b>LAB/BRANCH</b> Endocrinology and Reproduction Research Branch		
<b>SECTION</b> Section on Hormonal Regulation (Endocrine Physiology Unit)		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS:</b>  2.0	<b>PROFESSIONAL:</b>  1.5	<b>OTHER:</b>  0.5
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) <p>Current investigation in this project has focused on the regulation and actions of corticotropin releasing factor (CRF) receptors, and the interactions of CRF with other ACTH regulators including vasopressin (VP), angiotensin II (AII), norepinephrine and glucocorticoids.</p> <p>A. Pituitary CRF receptor regulation. We have previously shown that the increases in plasma ACTH after adrenalectomy are accompanied by pituitary CRF receptor down-regulation and desensitization. Further studies in rats receiving CRF infusion demonstrated that sustained exposure of the pituitary to CRF causes CRF receptor loss and a specific decrease in CRF-stimulated adenylate cyclase activity, which could partially account for the changes following adrenalectomy.</p> <p>B. CRF receptors in the nervous system. Similar to previous findings in the rat, studies in the monkey demonstrated the presence of CRF receptors in the cerebral cortex and limbic system related areas in the primate brain. In the peripheral nervous system, the importance of CRF receptors in the adrenal medulla was emphasized by studies in isolated bovine chromaffin cells which demonstrated the ability of CRF to stimulate catecholamine and met-enkephalin secretion.</p> <p>C. Interactions between ACTH regulators and mechanism of action. In addition to the cyclic AMP-dependent mechanisms by which CRF stimulates the corticotroph, activators of protein kinase C such as phorbol esters, synthetic diacylglycerol and phospholipase C were found to stimulate ACTH secretion. This effect was additive to the stimulatory effect of CRF, but not to those of VP, AII and norepinephrine, suggesting the involvement of protein kinase C in the action of cyclic AMP-independent stimuli. In regard to glucocorticoid feedback, experiments in isolated pituitary cells demonstrated that the biphasic inhibitory pattern of ACTH secretion observed <u>in vivo</u> also occurs <u>in vitro</u> in the corticotroph. The two inhibitory components have different kinetics and sensitivity to corticosterone and probably involve different mechanisms of action of glucocorticoids in the corticotroph.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00192-01 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Purification, Immunology and Functional Activity of Adrenocortical Proteins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. A. Strott Head ERRB, NICHD

Others: Y. C. Lee Sr. Staff Fellow ERRB, NICHD  
C.D. Lyons Bio. Lab. Tech. ERRB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Adrenal Cell Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.75

## PROFESSIONAL:

1.25

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of non-catalytic proteins in the adrenocortical steroidogenic process is vague and speculative. Non-catalytic proteins have been implicated in a variety of ways such as regulation of cholesterol side-chain cleavage activity (the rate-limiting step in steroidogenesis), cholesterol and pregnenolone transport mechanisms, secretory processes, etc. However, to date, no non-catalytic protein has been completely isolated and characterized for the adrenal cortex. The elusive steroidogenic regulatory protein remains to be identified. The adrenal cortex of the guinea pig contains specific steroid-binding proteins which have been only partially purified and characterized; their function is as yet undetermined. For instance, there are proteins which specifically bind cholesterol, cholesteryl sulfate, pregnenolone, and pregnenolone sulfate. These proteins are of great interest because the rate-limiting reaction in steroidogenesis is the conversion of cholesterol to pregnenolone or cholesteryl sulfate to pregnenolone sulfate. The pregnenolone-binding protein, which is isolated from the high speed soluble fraction, has been purified to the greatest extent, but the best current preparations still contain numerous contaminants. To further purify the pregnenolone-binding protein, two approaches are currently being used: 1) development of an affinity probe, 2) generation of antibodies to proteins electroeluted from sodium dodecyl sulfate gel slices. Several antisera have now been generated which are presently being examined by Western blot analysis as well as for interaction with the pregnenolone-binding protein using the technique of sucrose density gradient analysis. It is planned in the near future to obtain the capability to utilize high performance liquid chromatography for purifying isolated gel protein bands. In addition to purifying steroid-binding proteins, it is becoming increasingly apparent that selective phosphoproteins will need to be isolated and characterized. The latter task will be difficult and time-consuming, but will be necessary if a specific functional significance is to be ascertained for a particular phosphorylated (or dephosphorylated) protein.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00193-01 ERRB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Angiotensin II Receptors and Activation Mechanisms

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K.J. Catt	Head	ERRB, NICHD
Others:	G. Aguilera	Research Biologist	ERRB, NICHD
	G. Guillemette	Guest Researcher	ERRB, NICHD
	A. Baukal	Biomedical Engineer	ERRB, NICHD
	T. Balla	Visiting Fellow	ERRB, NICHD
	C. Harper	Guest Researcher	ERRB, NICHD
	M. Carson	Guest Researcher	ERRB, NICHD
	W. Hausdorff	Guest Researcher	ERRB, NICHD

## COOPERATING UNITS (if any)

Dept. of Physiology, Semmelweis University Medical School, Budapest (A. Spat)  
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

## LAB/BRANCH

Endocrinology and Reproduction Research Branch

## SECTION

Section on Hormonal Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

5.0

## PROFESSIONAL:

3.0

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The properties of angiotensin II (AII) receptors were studied in the adrenal zona glomerulosa, and the mechanisms leading to stimulation of steroidogenesis were analyzed in isolated glomerulosa cells from the rat and bovine adrenal cortex. AII receptors were further characterized by photoaffinity labeling with a C-terminal azido AII derivative, which possessed high labeling efficiency and was applied to the analysis of receptors in several target tissues. Isolation of the photolabeled AII receptor of the bovine adrenal gland was pursued by detergent solubilization and fractionation by ion exchange, lectin-affinity, and immunoaffinity chromatography. Studies on the actions of AII revealed that the calcium channel agonist, BAY K 8644, increased basal aldosterone production and enhanced the responses to AII and  $K^+$  in a differential manner, by increasing the maximum aldosterone response to AII but not to  $K^+$ . These findings suggest that voltage-sensitive calcium channels are partially operative under basal conditions and are further activated by AII and  $K^+$ . Elevation of cytoplasmic calcium by AII also depends upon mobilization of intracellular calcium stores by the products of ligand-stimulated phosphoinositide turnover. Microsomal receptors for the putative mediator of calcium mobilization, inositol-1,4,5-trisphosphate ( $IP_3$ ) were identified in adrenal microsomes by binding studies with [ $^3P$ ]  $IP_3$ , and show high specificity and affinity ( $K_d$  5 nM) as well as low capacity for  $IP_3$ . The generation of inositol-1,4,5-trisphosphate from phosphatidylinositol bisphosphate during AII action was extremely rapid and was accompanied by major production of  $IP_2$  and inositol-4-monophosphate as well as formation of the inactive  $IP_3$  isomer, inositol-1,3,4-trisphosphate. The finding that  $IP_3$  is rapidly degraded to Ins-4-P contrasts with the previous view that Ins-1-P is the major metabolic product, and indicates that Ins-4-P serves as a marker of polyphosphoinositide turnover. The  $IP_3$ -receptor system and the activation of voltage sensitive calcium channels are the major mechanisms involved in the regulation of intracellular calcium by AII and potassium, respectively, in the adrenal zona glomerulosa cell.







## HUMAN GENETICS BRANCH

- Z01 HD 00131-12 Human Biochemical Genetics  
William A. Gahl, M.D., Ph.D.
- Z01 HD 00133-09 Study of Glycogen Storage Disease  
James B. Sidbury, Jr., M.D.
- Z01 HD 00403-05 Magnesium Metabolism in Mothers and Neonates  
Joan L. Caddell, M.D.
- Z01 HD 00404-04 Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases  
Jean DeB. Butler, Ph.D.
- Z01 HD 00405-08 Structure of the Methionine Initiator tRNA Genes in the Human Genome  
Michael A. Zasloff, M.D., Ph.D.
- Z01 HD 00408-03 Pathophysiology and Treatment of Human Genetic Diseases  
Michael A. Zasloff, M.D., Ph.D.
- Z01 HD 00410-01 Metabolism in Children with Glycogen Storage Disease, Type I  
James B. Sidbury, M.D.
- Z01 HD 00411-01 Evaluation of Nalmefene, an Endorphin Antagonist, in the Control of Appetite  
James B. Sidbury, M.D.
- Z01 HD 00909-07 Effects of Ethanol on the Mother and the Fetus  
Anil B. Mukherjee, M.D., Ph.D.
- Z01 HD 00910-07 Uteroglobin: Physiological Function and Genetic Regulation  
Anil B. Mukherjee, M.D., Ph.D.
- Z01 HD 00912-07 Gene Regulation and Cellular Differentiation  
Janice Y. Chou, Ph.D.



NICHD Annual Report  
October 1, 1985 to September 30, 1986

Human Genetics Branch

The Human Genetics Branch conducts research which attempts to elucidate the pathophysiology of human genetic and developmental disorders through an understanding of basic biological mechanisms. Clinical activities include studies of the natural history, treatment, and methods of diagnosis of several heritable disorders of man.

Section on Molecular Biology

During the past year, work in the section directed by M. Zasloff has concentrated on several problems: the mechanism of processing of eukaryotic RNAs; the basic cellular mechanism involved in the transport of RNA from nucleus to cytoplasm; the expression of ALU sequences in eukaryotes. Clinical research in the Section has focused on processes and disorders related to bone formation in man; the pathophysiology of osteogenesis imperfecta; the pathophysiology and treatment of fibrodysplasia ossificans progressiva; the expression of collagen genes in man; the structure of the bone-specific alkaline phosphatase gene in man.

Over the past several years, this group has studied the pathway of expression leading to biosynthesis of the tRNA<sup>met</sup><sub>1</sub> molecule in human cells. The studies have provided insights into the organization of the tRNA gene family in man and the pathways utilized in delivery of a mature tRNA into the cytoplasm of eukaryotic cells. The laboratory had previously shown that a naturally occurring tRNA<sup>met</sup><sub>1</sub> variant exhibited a defected phenotype in several in vitro systems. The group demonstrated that the natural variant gene contained a point mutation which resulted in the appearance of a primary gene transcript that was inefficiently processed. In addition, through the use of microinjection and micro-dissection methodology in the *X. laevis* oocyte system, the group demonstrated that the variant tRNA was defective in its transport from the nucleus to the cytoplasm. This discovery led to the first description of the mechanism by which an RNA is transported from the nucleus of a eukaryotic cell. It was demonstrated, in fact, that tRNA species were transported by a saturable, carrier-mediated mechanism. We proposed that a ribosome-like element at the nuclear envelope was the actual motor utilized in this process. To fully explore the domain of the tRNA<sup>met</sup><sub>1</sub> molecule recognized by the transport system, 30 point mutations were generated in the human gene by in vitro mutagenesis utilizing hydroxylamine. Analysis of the transport phenotypes obtained demonstrated the unexpected finding that the tRNA molecule is exquisitely sensitive to mutation in its transport properties. The particularly sensitive areas included the T and D loops of the tRNA, the most highly conserved portions of the species. Another feature of this study was the demonstration that every mutation which yielded a tRNA species defective in transport, also yielded a pre-tRNA species which was inefficiently processed in vivo. This correlation led to the postulate that the processing enzymes might in some manner be playing a role in tRNA transport. As a result, the processing nucleases were purified from eukaryotic cells, representing the first characterization of these classes of enzyme in eukaryotes. We demonstrated that two enzymes, both endonucleases, process the primary transcript of the human tRNA<sup>met</sup><sub>1</sub> gene to a mature species. The first reaction involves cleavage of the 5' leader; the second, cleavage of the 3' trailer. Two enzymes have been purified from *X. laevis* oocytes and KB cells. The enzyme

which processes the 3' trailer is a simple polypeptide of about 97,000. Its most striking feature is that it will only cut the 5' processed primary transcript, establishing a cutting order to the pathway. The first cutting activity falls into the lap of the 5' nuclease. This enzyme has been purified to homogeneity and appears to be one of the most complex enzymes yet described in animal cells. It is composed of at least 14 different polypeptides ranging in MW from 20,000 to 32,000. It has the shape of a cylinder, composed of a stack of 4 rings. The entire structure appears to be necessary for tRNA processing. The precise relationship between its structure and enzymatic activity is under study. It is curious that this particle has been described in the literature over the past 15 years, having been isolated from organisms ranging from *Drosophila* to man. Until our report, its function remained a mystery. The relationship between this particle and tRNA transport remains to be ascertained.

Work began over the year to define the mechanism of mRNA nuclear transport, a fundamental eukaryotic process about which virtually no solid data exists. The system utilized has been the *X. laevis* oocyte, with the mRNA being the Herpes TK gene transcript. This product is efficiently synthesized and functional enzyme can be assayed within several hours of gene microinjection, demonstration of the integrity of the entire expression pathway for this gene in the *X. laevis* oocyte. We developed new methodology to study mRNA translocation and have defined the intracellular distribution of RNA following gene introduction. The most striking finding is that mRNA remains intranuclear for about 90 minutes at which time all RNA transcribed from the TK gene is suddenly quite efficiently transported. Virtually no steady state levels of TK sequence accumulate in the nucleus after 90 min. The questions to be answered next involve the structural features of a eukaryotic mRNA which perturb movement between compartments. The role of translation punctuational information in mRNA movement will be explored utilizing appropriate in vitro constructs.

We have continued our study of the Alu sequence family over the past year. These sequences comprise around 3-6% of the vertebrate genomes, present in about 300,000 copies. We have demonstrated that one such sequence, the murine B1 sequence, is processed and transported from nucleus to cytoplasm. The particular sequence studies lies antisense to a murine gene encoding alpha-fetoprotein (AFP). The processing reaction involving the Alu primary transcript appears to be endonucleolytic, releasing the 3' trailer. The "core" Alu RNA generated is transported upon processing into the cytoplasm. We have shown that this species is present in highest abundance in murine fetal liver, the tissue in which AFP is most actively expressed. This result was unexpected since current thinking would assign an inhibitory role to this natural antisense RNA. The Alu sequence RNA, in addition, was shown to be associated with a specific polypeptide of about 63,000 in MW, a polypeptide identified through the use of an autoimmune antiserum from a patient with lupus. This polypeptide appeared to be associated with the primary transcript of the Alu sequence in its nuclear phase, and remained associated after processing and transport to the cytoplasm. The affinity purified antibody further identified a protein of similar size in KB cells. Associated with this polypeptide in the human cells was a small RNA of about 80 nt. We suspect that this RNA will represent the human analogue of the B1 Alu family. It suggests that despite nucleic acid sequence divergence, the Alu sequence between vertebrates may be functionally conserved. Its role in gene expression in several systems is under study. The pathophysiology of hypophosphatasia was investigated at a molecular genetic level. The initial approach has been to isolate the cDNA and gene encoding boneto homogeneity from liver



and partial sequence information obtained. Appropriate synthetic oligonucleotides were generated and cDNA libraries are in the process of being screened for potential AP clones. With these probes, the basic defect in this disorder of ossification will be explored.

Studies on the pathophysiology and treatment of osteogenesis imperfecta continue. After considerable effort, the transcription units for alpha-2 type I collagen was determined in both skin and osteoblasts of avian and human origin. In both cases the mRNAs do not undergo alternative splicing or variations in promoter usage in bone and skin fibroblastic cells. This result suggests that other mechanisms must operate to explain the considerable greater abundance of collagen-specific mRNA in the osteoblast, including tissue-specific enhancers, splicing alternatives 3', differential stability, etc.

Techniques for identification of mutation in the collagen polypeptide genes were continued. These included mRNA/DNA mismatch detection, polypeptide synthesis in culture, thermal stability of secreted procollagen, and restriction length polymorphism. It is expected that as mutations are identified they will be used to extend our understanding of the role of collagen structure in bone development.

Clinical studies in O.I. have dealt with the use of lower limb bracing in children with O.I. and the hormonal basis of growth failure in certain affected children. The bracing study has asked whether support of the lower limbs accelerates walking in affected children. This is important since weight bearing leads to enhanced mineralization and more dynamic modeling of the fragile lower limb skeleton. Initial results are exciting and will almost certainly lead to the application of these bracing techniques in this disorder. The second study attempts to determine if a defect in the known growth-promoting hormones is correlated with growth failure in O.I. It appears that growth failure is seen variably in affected individuals, unrelated to severity of bone fragility. The current studies involve evaluation of growth hormone secretion and IGF I and IGF II levels, both steady state and provoked. Initial studies suggest that children with severe O.I. may have extremely low levels of circulating IGF I, suggesting a role of this hormone in the process. The apparent decrease in GH secretion in some affected patients has prompted the use of clonidine as a stimulant of endogenous secretion.

Studies on fibrodysplasia ossificans progressiva continue. FOP is a rare inherited disorder of heterotopic ossification characterized by relentless appearance of mature enchondral bone around skeletal muscle. Treatment of the disease with Accutane enters its 3rd year, with results quite promising. Initial review of our data, based on about 10 patients treated and about 5 controls, suggests that Accutane arrests new bone formation at a dose of about 5 mg/kg/day. The full study will be collated in the coming year. Several clinical diagnostic studies were completed demonstrating the use of Tc99M and CAT scan procedures in the diagnosis and management of FOP. With respect to pathophysiology, we demonstrated this past year that all patients with FOP possess a circulating prostanoid which cross-reacts with PGE2 by radioimmunoassay. This compound appears to be present in patients' plasma at between 10-100 fold higher levels than unaffected individuals. The exact nature of the compound is being determined at the present time through mass spectroscopic analysis. We suspect that it represents a stable, well described metabolite. If so, it will suggest that prostanoid synthesis in these patients is markedly accelerated. If it represents a new prostanoid, the structure of the compound may suggest it to

be an inducer itself. Catheterization of a female with active bone formation this past year revealed that this prostanoid is not secreted by the tumors itself.

A new disorder of heterotopic ossification in children was described this past year. It appears to represent a disorder of dermal differentiation and follows a dramatically different natural history than FOP. Potential mechanisms have been proposed.

#### Section on Disorders of Carbohydrate Metabolism

The study of the response of patients deficient in glucose 6-phosphatase to the administration of different raw starches continues. With rare exceptions, corn starch continues to be the most efficacious in terms of sustaining a normal blood glucose level over a 6-hour period. Studies have been undertaken to utilize the serum amylase to detect heterogeneity and to determine whether a particular child under the age of 4 years has sufficient pancreatic amylase to be a candidate for starch therapy. The liver glucose production rates have been determined in 4 patients with type I glycogen storage disease and one with type III. We have verified the production of glucose by individuals with deficient glucose 6-phosphatase activity. We can distinguish the rate of production in those with total absence from those with partial deficiency.

An assessment of the potential efficacy of nalmefene, a third general compound, in the control of appetite in the Prader-Willi syndrome and found it totally wanting.

A study of infants with idiopathic apnea neonatorum admitted at 6 weeks or 10 weeks after both were alternately treated with magnesium. Those treated had fewer recurrences. Mice made magnesium-deficient and subjected to an audiogenic or strychnine seizures developed lung pathology similar to that found in hyaline membrane disease when studied by routine histology and electromicroscopy. Normal mice did not show such pathology.

#### Section on Developmental Genetics

This Section conducts research to understand (1) the physiological role and genetic regulation of endogenous phospholipase A2 inhibitory proteins such as uteroglobin and lipocortins and (2) the mechanism of intrauterine growth retardation and the genetic predisposing factors in Fetal Alcohol syndrome (FAS).

During the past year we have demonstrated that uteroglobin (Utg), a steroid-dependent small molecular weight secretory protein in the rabbit, is a potent inhibitor of phospholipase A2 (PLA2) activity. Utg has been found to inhibit PLA2 both in cultured cells as well as in a cell-free system. Since PLA2 activity is required to generate arachidonate, the substrate for prostaglandin (PG) synthesis, experiments were carried out to delineate whether Utg inhibited cellular prostaglandin levels. To study this and the regulation of the Utg gene expression, cell lines from all organs of the rabbit where Utg is found have been established by transformation with SV40 tsA mutant. Two such cell lines derived from the adult rabbit endometrium (RBE-7 and H5DC) and one cell line derived from young rabbit endometrium (YRE-1) have so far been characterized. At 39.4°C, when stimulated with progesterone ( $10^{-9}$ M) after 4 days of estradiol ( $10^{-9}$ M) priming, an increased rate of transcription as well as



translation of the Utg gene occurs in these cells. These cells, when stimulated with estradiol or phorbol ester, were found to have a high PLA2 activity and consequent high level of prostanoids (PGE2, PGF2 $\alpha$ , 6KPGF $\alpha$  and thromboxane B2). These cells when further treated with exogenous Utg (20 $\mu$ M) a 78% reduction in PLA2 activity occurred with an 85% inhibition in prostanoid levels as compared to control values. To determine whether endogenous Utg production by cells would reduce PLA2 activity, we treated the phorbol ester pretreated cells with progesterone (10<sup>-9</sup>M) for 24 hours when Utg production was detected. Significant reduction in PLA2 activity as well as lowered prostanoid levels (as measured by HPLC & RIA) were observed. This reduction of PLA2 activity and/or prostanoid levels were not observed when either cyclohexamide, a protein synthesis inhibitor, or a monospecific antibody to Utg was added following progesterone treatment of these cells. The YRE-1 cell line behaved similar to RBE-7 and H5D:C cells when the same experiments were repeated. Preliminary in vivo experiments seem to confirm the in vitro results.

Last year, preliminary results for the detection of a human protein similar to Utg were presented. Further experiments have confirmed that there is a small molecular weight secretory protein present in the human tracheobroncheal and endometrial epithelium during midluteal phase of the cycle.

In collaboration with Drs. Bryan Gowan, University of Mississippi and Howard Zacur, Johns Hopkins Hospital, we have studied endometrial washings and endometrial tissues from 48 women at different stages of the menstrual cycle. It was confirmed that Utg-like protein appears in the midluteal phase of the human endometrium. Additional studies are now in progress to determine whether there is any correlation between the presence or absence of this protein and habitual abortion. Two human endometrial cell lines have been established with SV40 transformation. These cells are now being characterized for the production of Utg-like protein. If enough protein is produced by these cells, an antibody will be raised and establishment of cDNA clone for the human gene will be attempted.

To delineate the active site of the Utg molecule involved in PLA2 inhibition, sitedirected mutagenesis of the Utg gene and its expression in bacterial vector have been undertaken this year. These mutated proteins will then be tested for PLA2 inhibiting activity. Studies have begun on the expression of the Utg gene in *E. coli*. A full-length cDNA containing the entire pre-Utg coding region was obtained from Dr. David Bullock (Baylor College of Medicine, Houston, TX). From this cDNA, a 490 base pair Pst-I fragment was prepared. This fragment contains the entire mature coding region, starting with Gly-1 codon. This fragment was subcloned in Pst-I site of the expression vector pKK233-3 (kindly supplied by Dr. J. Brosius, Columbia University, NY). The orientation of the insert was checked by restriction digestion and one of the positive clones (pLE-101) was tested for protein expression. In this expression system the cDNA insert is under the control of the synthetic "trc" promoter, which in turn is controlled by the lac repressor protein when lac I<sup>q</sup> *E. coli* host is used. Two different lac I<sup>q</sup> hosts were used, e.g., JM-105 and JM-109 (obtained from Dr. J. Messing, University of Minnesota, Minneapolis, MN). After transformation with pLE-101 and colony purification, single Amp<sup>r</sup> colonies were grown in LB-Amp medium up to 0.7 OD<sub>650</sub> and induced with the gratuitous inducer isopropyl-thiogalactopyranoside (IPTG) at a final concentration of 1mM. After 1 hour, bacteria were harvested and Utg production was tested by RIA on aliquots of bacterial lysates. Uninduced controls were run in parallel. The level of

Utg production was 1.25  $\mu\text{g/gm}$  of cells for JM-105 and about 5  $\mu\text{g/gm}$  of cells for JM-109. Since the purpose of these experiments was to obtain large amounts of purified recombinant Utg, these levels of expression are considered too low. The two main reasons for low expression of eukaryotic proteins in *E. coli* are proteolysis and/or low efficiency translation. Therefore, two new expression systems based on the lambda-PL promotor will be tested: pAS<sub>1</sub> (obtained from Dr. A. Shatzman, Smith, Kline and French) and pRC23 (a generous gift from Dr. R. Crowl, Hoffman-LaRoche). These vectors contain synthetic strong ribosome binding sites that assure efficient translation of the insert upon induction by temperature shift. Moreover, protease deficient (*lon*<sup>-</sup>) *E. coli* strains will be used to minimize protein loss due to proteolysis.

The role of genetic predisposing factors in developing fetal toxicity of ethanol is continued. During the past year we have studied the transketolase abnormality in cultured fibroblasts from a diabetic patient who developed Wernicke's encephalopathy when treated with tolazamide, a hypoglycemic agent. Additionally, three diabetic kindreds without any history of Wernicke's encephalopathy and four normal controls were also studied. We found that the patient with tolazamide-induced Wernicke's encephalopathy and one of the three diabetic kindreds had abnormal  $K_m$  of transketolase for TPP similar to the ones found previously in post-alcoholic Wernicke's Korsakoff syndrome. These data suggest that transketolase abnormality is prevalent in the population and the individuals with this abnormality may be predisposed to developing thiamine deficiency syndromes either by drugs that increase thiamine utilization or by ethanol abuse. To investigate whether or not this enzyme abnormality is associated with FAS, we studied 2 cultured amniotic fluid cell lines where the neonates were diagnosed to have FAS by several criteria. Three control amniotic fluid cell lines from normal pregnancies were also investigated. Preliminary results suggest that both amniotic cell lines with FAS pregnancies have abnormal transketolase enzyme as suggested by the determination of  $K_m$  of this enzyme for TPP and compared to control values. Studies in progress will investigate the cells from more FAS, normal control pregnancies as well as cells derived from pregnancies where mother had a history of alcohol abuse, but the baby was unaffected. In a related study, we have investigated the transketolase enzyme kinetics in fibroblasts from ten members representing three generations of an Amish family. The results show a pattern of autosomal recessive type of inheritance of the abnormality as previously speculated by McKusick. We have recruited three patients with FAS in our clinical protocol to search for a genetic factor(s) in FAS as well as to study the pathophysiology of this common disorder.

#### Section on Human Biochemical Genetics

The Section on Human Biochemical Genetics investigates the clinical and basic research aspects of inborn errors of metabolism in man. Its pursuits have been diverse and intensive.

The cause of a rare lysosomal storage disease was documented in the past year. Salla disease, a Finnish disorder characterized by psychomotor retardation and lysosomal accumulation of free sialic acid, was shown to be caused by a deficiency of normal free sialic acid transport across the lysosomal membrane. This makes Salla disease the second storage disorder due to impaired transport of a small molecule out of lysosomes, and sialic acid the first monosaccharide for which a carrier-mediated transport system within the lysosomal membrane has been described. Three years ago, the Section reported that cystinosis was the first



lysosomal storage disorder due to a transport deficiency (for cystine); these two discoveries have created a new field in the area of human metabolism, with descriptions of similar disorders likely to follow.

Work by the Section has employed the technique of counter-transport to demonstrate the existence of a new lysosomal transport system for tyrosine and other neutral amino acid in cultured rat thyroid cells. The carrier system resembles the L system described in plasma membranes. The rat thyroid cells were also shown to possess a lysosomal cystine carrier closely resembling that of human leucocyte and fibroblast lysosomes. The rat carrier system exhibited a half-life of approximately 24 hours when cells were cultured in the presence of the protein synthesis inhibitor cycloheximide. Total genomic DNA from the rat cells will be used to transfect human cystinotic fibroblasts, followed by a selection technique that kills the mutant, but not the "cured" cells.

Cultured fibroblasts from patients with mucopolipidosis II (I-cell disease), which store free cystine in their lysosomes, were shown to lack normal amounts of lysosomal cystine transporting capacity. In similar experiments, cells from a patient with intermediate or late-onset cystinosis exhibited no lysosomal cystine egress, just as in nephropathic cystinosis cells. However, leucocytes from a patient with benign cystinosis, who had no renal disease and limited cystine storage, displayed a significant amount of residual lysosomal cystine-carrying capacity. The severity of clinical disease in cystinosis may be related to the degree of residual cystine carrier as well as other genetic and environmental factors.

The Section has vigorously pursued a putative defect in glycosaminoglycan sulfation in Lowe (oculocerebrorenal) syndrome, demonstrating normal synthesis and sulfation of proteoglycans in this X-linked disorder of mental retardation, congenital cataracts, and renal Fanconi syndrome. Both cultured fibroblasts and muscle cells were examined in these studies.

In clinical studies, the largest group of nephropathic cystinosis patients in the United States and Canada (approximately 25) have been treated with the cystinedepleting agent, cysteamine, for the past 8 years. A significant improvement in growth and a retardation of renal deterioration have been appreciated. At the same time, serious post-renal transplant complications of cystinosis have been documented, including diabetes mellitus, cerebral atrophy and calcifications, and ocular abnormalities such as blindness, posterior synechiae and corneal erosions. These findings have prompted the initiation of cysteamine therapy for post-transplant cystinosis patients and the performance of corneal transplant in one 12-year-old boy with debilitating corneal erosions.

Other ongoing therapeutic protocols involve oral L-carnitine repletion for carnitine-deficient patients with renal Fanconi syndrome and betaine therapy for pyridoxine-nonresponsive homocystinuric patients. In addition, a 31-year-old man with hypermethioninemia was diagnosed as having hepatic methionine adenosyltransferase deficiency. Prior to this, the enzyme deficiency was described only in young children. Consequently, this individual adds 25 years of natural history to our current knowledge of the disorder in man. He also represents an example of the type of rare disorder the Section continues to investigate to gain insight into the causes and effects of metabolic deficiencies in man.

The studies in the Section directed by Janice Chou have concerned regulation of

gene expression during normal and abnormal differentiation processes. Studies on the expression of the alpha-fetoprotein (AFP) gene in temperature-sensitive (ts) fetal liver cells were continued. It has been demonstrated that transformed fetal hepatocytes synthesized a variant AFP of 65K which was encoded by a mRNA of 16S. However, differentiated fetal hepatocytes synthesized two AFPs of 73K and 69K, which were encoded by a mRNA of 20S. Hybridization experiments demonstrated that the two mRNAs differ in sequences at the 5' end. Nuclease S1 mapping experiments showed that the 16S mRNA lacked the first six exons, Z, A, B, C, D, and E which were normally present in the 20S RNA. Since there is only one AFP gene per haploid genome in rat, the two AFP mRNA species may be generated by the use of different promoters.

In studies on hormonal regulation of gene expression, Chou and her colleagues found that glucocorticoid hormone is a potent agent that induces fetal maturation. They studied regulation of expression of specific fetal and adult genes in the ts fetal hepatocyte line established in her laboratory. At 40°C, these cells exhibit a differentiated phenotype, glucocorticoid hormone suppressed expression of the fetal gene, AFP, but induced expression of the TAT gene. TAT is an enzyme that appears only postnatally. Thus, fetal liver cells matured into adult hepatocytes in the presence of glucocorticoid hormone.

To ascertain how closely the ts hepatocyte systems resemble primary liver cells, Chou's group studied the ability of the adult hepatocytes to express a large number of liver-specific functions. In addition to expressing genes encoding AFP, albumin, and transferrin, they found these cells express genes encoding TAT, phosphoenolpyruvate carboxykinase (PEPCK), and fibrinogen. As expected, expression of these genes was ts and expressed mainly at the nonpermissive temperature of 40°C. At 40°C, these cells exhibit a normal differentiated phenotype. Most importantly, expression of all of these genes was dependent upon the presence of glucocorticoid hormone. In collaboration with Dr. G. Yeoh of Western Australia, they studied the expression of TAT gene in detail. They found that transformed hepatocytes synthesized low levels of TAT, but differentiated cells synthesized greatly increased amounts of this enzyme. Glucocorticoid hormone was absolutely required to sustain TAT expression. Differentiated hepatocytes synthesized no detectable levels of TAT in the absence of glucocorticoid. cAMP enhanced the induction by glucocorticoid, but cannot replace this steroid hormone. TAT synthesis was not detectable in differentiated hepatocytes in the presence of cAMP alone. Furthermore, they demonstrated that the change in TAT synthesis paralleled the change in TAT mRNA levels.

Studies on the expression of the human placental alkaline phosphatase (PAP) gene at the molecular level were continued. They found that different PAP mRNAs were expressed in choriocarcinoma and HeLa cells as compared to human placenta. Choriocarcinoma cells are malignant trophoblasts which produce PAP eutopically whereas HeLa cells produce this phosphatase ectopically. PAP mRNA of placenta migrated as a 2.7-kb band, whereas PAP mRNAs from choriocarcinoma and HeLa cells migrated as 2.4-kb and 2.6-kb bands, respectively. Sodium butyrate induced the biosynthesis of PAP and increases the PAP mRNA levels in both types of cells. HeLa PAP synthesis was induced also by prednisolone. The apparent molecular weight of the PAP monomer in both type of cells in the presence or absence of inducers was similar. In choriocarcinoma cells, sodium butyrate increased the levels of the 2.4-kb PAP mRNA. However, in HeLa cells, Prednisolone induced the expression of the 2.7-kb PAP mRNA whereas butyrate induced the expression of two mRNAs for PAP, the 2.7-kb band and a fast migrating band at 2.5-kb.

Analysis of the mRNA and genomic structure of PAP should yield information as to its polymorphism.

Studies of the expression of the pregnancy-specific  $\beta$ -glycoprotein (PS $\beta$ -G) gene at the molecular level have been extended over the past year. Genes encoding PS $\beta$ -G were cloned. Efficient isolation of these genes was accomplished by probing a phage lambda gt11 expression library with antibodies to PS $\beta$ -G. The identity of the cDNA clones was confirmed by comparing the predicted amino acid sequences to sequences determined from fragments of placental PS $\beta$ -G. These probes will be used to examine molecular mechanisms regulating PS $\beta$ -G synthesis in placental and fibroblasts.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 00131-12 HGB
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders )</b> Human Biochemical Genetics		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>		
PI:	William A. Gahl	Medical Officer                      HGB, NICHD
Others:	Isa Bernardini	Technician                      HGB, NICHD
	Juan Bernar	Medical Staff Fellow              HGB, NICHD
	Gregory Harper	Visiting Fellow                  HGB, NICHD
	Martin Renlund	Guest Researcher                HGB, NICHD
<b>COOPERATING UNITS (if any)</b> See Attached		
<b>LAB/BRANCH</b> Human Genetics Branch		
<b>SECTION</b> Section on Human Biochemical Genetics		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
4.0	3.0	1.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> (1) We demonstrated defective lysosomal cystine transport in leucocytes and fibroblasts from patients with the intermediate (juvenile) and benign (adult) variants of cystinosis. In other studies, the cystine carrier was functional in rat thyroid-derived cells, and required protein synthesis but non N-linked glycosylation for its production. (2) Twenty-five children with cystinosis contributed data toward a national study demonstrating the efficacy of oral cysteamine therapy in enhancing growth and retarding renal failure. At the same time, late complications of cystinosis were described, including cerebral atrophy, impaired salivary function, diabetes mellitus, restrictive pulmonary function, corneal erosions, and reduced visual acuity. One patient received a corneal transplant, and a protocol for treating corneal erosions with cysteamine eyedrops was initiated. Oral cysteamine is being offered to post-renal transplant patients. Carnitine-deficient individuals with Fanconi syndrome are being treated with oral carnitine with some success in normalizing their muscle histology. (3) The second lysosomal storage disorder due to defective transport of a small molecule across the lysosomal membrane is Salla disease, a Finnish disease characterized by psychomotor retardation. Salla fibroblasts store free sialic acid within their lysosomes due to impaired egress of the charged sugar. Egress velocity of sialic acid out of normal lysosome-rich granular fractions increased with increasing loading and temperature ( $Q_{10}=2.3$ ). (4) Lowe (oculocerebrorenal) syndrome fibroblasts manifested normal rates of hyaluronic acid and proteoglycan synthesis and sulfation, with a large degree of variability among normals. (5) Using counter-transport, a lysosomal transport system for tyrosine and other neutral amino acids was characterized for rat thyroid cells in culture. The system, with a $Q_{10}$ of 1.9 and apparent $K_m$ for tyrosine of 100 $\mu$ M, resembles the plasma membrane L system. (6) I-Cell (Mucopolipidosis II) fibroblasts demonstrated impaired lysosomal clearance of cystine. Pyridoxine-nonresponsive homocystinuric patients are treated with betaine to study its effect on bone density.		

## Cooperating Units:

F. Tietze, NIDDK  
S. H. Mudd, NIMH  
J. Schneider, University of California at San Diego  
J. Thoene, University of Michigan  
G. Thomas, Johns Hopkins University  
N. Bashan, Beersheva, Israel  
R. Helfgott, CC, NIH  
W. Rizzo, Medical College of Virginia  
M. Kaiser-Kupfer, NEI  
H. Levy, Massachusetts General Hospital  
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B. Wolf, Medical College of Virginia  
J. Hoofnagle, NIDDK  
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P. Backlund, NIMH  
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B. Fivush, Johns Hopkins Medical Center  
C. Porter, George Washington University Medical Center  
R. Chesney, University of California, Davis  
G. Ruley, George Washington University Medical Center  
G. Merriam, NICHD  
A. Tangerman, Nijmegen, The Netherlands  
G. Rodgers, Gladstone Laboratories, San Francisco, CA  
J. Williams, Univ. Texas, Houston  
J. Fink, NINCDS  
L. Kohn, NIDDK  
E. Grollman, NIDDK  
O. Hurko, Johns Hopkins University

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00133-09 HGB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Study of Glycogen Storage Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James B. Sidbury

Head

HGB, NICHD

## COOPERATING UNITS (if any)

Pamela Brye RD, CC, NIH

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.3

## PROFESSIONAL

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

The study is designed to test the heterogeneous responses of different patients with glucose 6 phosphatase deficiency in hydrolyzing and absorbing glucose when administered raw starches from different sources. When divergent results are obtained, family studies are pursued. Further, the results obtained are applied to the management of the patients with glucose 6 phosphatase deficiency.

Attempts are underway attempting to incorporate the various starches into acrylamide gels and then determine whether the heterogeneity can be shown using serum from the patients.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 00403-05 HGB
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less Title must fit on one line between the borders.) Magnesium Metabolism in Mothers and Neonates		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Joan L. Caddell	Guest Researcher HGB, NICHD
Others:	James B. Sidbury	Head HGB, NICHD
<b>COOPERATING UNITS</b> (if any) Joan Blanchette-Mackie (NIADDK, NIH) Kathleen Snowden and Nathaniel Jackson (Small Animal Section, VR, NIH)		
<b>LAB/BRANCH</b> Human Genetics Branch		
<b>SECTION</b> Section on Disorders of Carbohydrate Metabolism		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
1.0	1.0	0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type Do not exceed the space provided ) <p>Magnesium deficiency was studied retrospectively in human infants and prospectively in experimental animals in order to evaluate its possible role in the pathogenesis of problems chiefly affecting premature human neonates of suboptimal economic status, who are at high risk for Mg deficiency. The problems include the sudden infant death syndrome (SIDS) and idiopathic respiratory distress syndrome (RDS).</p> <p>The near-miss for SIDS (near-SIDS) was studied from a group of 139 Mg-unsupplemented premature neonates who had idiopathic apnea neonatorum who were readmitted with apnea at <math>70 \pm 6</math> days of age, and from 20 infants (19 term, 1 premature) who suddenly developed apnea for the first time at <math>48 \pm 12</math> days of age (apnea of late onset). The sickest infants of both groups exhibited similar clinical and laboratory findings of a metabolic crisis, with acidosis. Mg supplementation was associated with fewer subsequent readmissions for apnea. A hypothesis concerning the possible role of Mg deficiency in SIDS is proposed.</p> <p>During a very short span of time, the weanling rat with acute Mg deficiency is extremely sensitive to minimal external stimuli and may suddenly develop a near-fatal or fatal shock episode. Lungs show findings of shock. Morphometric analysis and electron microscopy revealed changes compatible with RDS: gaps between endothelial cells, hemorrhage, and edema, and components of the hyaline membranes in the alveolar sacs and ducts, with early deposition of eosinophilic material. Such findings were not found in Mg-sufficient rats challenged with strychnine seizures. Both Mg-deficient rats with audiogenic seizures and Mg-sufficient rats with strychnine seizures showed similar levels of plasma corticosterone, ruling out the possibility of suboptimal protection from glucocorticoids in Mg deficiency shock.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00404-04 HGB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jean DeBrohun Butler Senior Investigator HGB, NICHD

Others: Anil B. Mukherjee Head, SDG HGB, NICHD  
Sondra Levin Guest Researcher HGB, NICHD

## COOPERATING UNITS (if any)

P. Pentchev, NINCDS; S. Padilla, EPA; F. Tietze, NIDDK

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Molecular Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL

1.0

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anomalies in cholesterol metabolism uncovered similar to:
  - a) Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification.
  - b) Niemann-Pick D cells which do not store cholesterol but do show a lack of cholesterol esterification.
2. Finalization of the study of the glutathione cycle during growth in cystinotic versus normal cells.
3. Characterization of cystinotic cell metallathionein present in a 2-fold excess in cystinotic versus normal fibroblasts.
4. Inhibition of phospholipase A<sub>2</sub> by uteroglobin.
5. Development of a cystinotic cell selection system based on the excretion of cysteine-cysteamine mixed disulfide into medium after treatment with pantethine by cystinotic cells and not found in medium from pantethine treated normal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 HD 00405-08 HGB

PERIOD COVERED  
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Structure of the Methionine Initiator tRNA Genes in the Human Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  
P.I.: Michael A. Zasloff, M.D., Ph.D. Head HGB, NICHD

Others: Samuel Adeniyi-Jones, M.D., Ph.D. Visiting Associate  
Janet A. Tobian, Ph.D. Staff Fellow HGB, NICHD  
Pilar de la Pena, Ph.D. Visiting Fellow HGB, NICHD  
Anthony Adams, B.S. Biologist HGB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH  
Human Genetics Branch

SECTION  
Section on Molecular Biology

INSTITUTE AND LOCATION  
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.0

PROFESSIONAL:

4.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies continue in the area of tRNA transport, biosynthesis, and processing the molecular biology of Alu sequences, and the mechanism of mRNA transport. We have in previous studies described the discovery of a mechanism by which tRNA molecules are transported from the nucleus of eukaryotic cells. The particular features of the tRNA molecule recognized by this transport system was defined by an analysis of 30 single point mutants generated in vitro in the human tRNA met 1 gene. The studies demonstrated that all mutations generating transport-defective species also generated pre-tRNA species which were inefficiently processed, suggesting that the processing nucleases might be playing a role in transport. The processing nucleases were purified to homogeneity. The 5' processing enzyme was found to be a complex enzyme composed of at least 14 different polypeptides and comprising a cylinder shaped particle. The particle appears to be identical to a ubiquitous subcellular particle previously described in the literature over the past 15 years. Studies on the biology of the Alu sequence have identified the first evidence that these ubiquitous genes generate processed cellular RNAs. The existence of a novel polypeptide which interacts specifically with the Alu RNA has been identified through the use of autoantisera from an individual with lupus. With this antibody, a corresponding polypeptide has been identified in human cells suggesting that analogues of the mouse B1 Alu sequence exist in man.

Studies on the mechanism of mRNA transport continue. The principal advance over the past year has been the establishment of a precise system utilizing the *X. laevis* oocyte to follow the kinetics of movement of mRNA from nucleus to cytoplasm. With this system the transport model we have previously proposed, utilizing a ribosome fixed at the nuclear envelope as the motor, is being tested.

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 HG 00408-03 HGB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiology and Treatment of Human Genetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Michael A. Zasloff, M.D., Ph.D.

Head

HGB, NICHD

Others: Joan Marini, M.D., Ph.D.

Medical Staff Fellow

HGB, NICHD

Kenneth Huttner, MD., Ph.D.

Medical Staff Fellow

HGB, NICHD

Gary Gottesman, B.A.

Howard Hughes Fellow

HGB, NICHD

Anthony Adams, B.S.

Biologist

HGB, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Molecular Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

4.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies at the basic and clinical level continue in several heritable bone disorders. Molecular genetic studies on the structure of the transcription unit of alpha 2 Type I collagen mRNA was completed. We demonstrated that in both dermal fibroblasts and osteoblasts identical splicing patterns and promotor usage occur in man and chick, suggesting alternative mechanisms must operate in tissue-specific regulation in different connective tissues. Studies began on the cloning of bone-specific human alkaline phosphatase, with the protein purified to homogeneity from human liver and a partial protein sequence data determined. Studies of the molecular defect in osteogenesis imperfecta continue with preliminary development of several new methods of delineating the collagen-specific subsets under way. Clinical studies in O.I. on the relationship of IGF I and II to growth failure show some promising correlations. Bracing as a treatment modality in childhood O.I. patients was continued. In the study of fibrodysplasia ossificans progressiva, treatment with 13-cis retinoic acid enters its 3rd year, with continued promise. The existence of a polar prostanoid in the plasma of patients with FOP has been confirmed and its identify at present is being determined. A new disorder of heteroptic ossification, limited dermal ossification, was described this past year.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 HD 00410-01 HGB
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Metabolism in Children with Glycogen Storage Disease, Type I		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	James B. Sidbury	Head HGB, NICHD
Others:	Joseph Muenzer Abraham Karkowsky	Medical Staff Fellow Medical Staff Fellow HGB, NICHD HGB, NICHD
<b>COOPERATING UNITS</b> (if any) Laboratory of Theoretical and Physical Biology (A. Yergey and N. Estaban)		
<b>LAB/BRANCH</b> Human Genetics Branch		
<b>SECTION</b> Section on Disorders of Carbohydrate Metabolism		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  This study was designed to determine the rate of glucose production by the liver in patients with absent glucose 6 phosphatase, deficient glucose 6 phosphatase and deficient translocase I as well as type III glycogenosis. There are reports that the liver of patients with type I glycogenosis produce some glucose. This interpretation was to be tested to determine whether there is a detectable difference in glucose production by the liver of those individuals who have a total absence of glucose 6 phosphatase in contrast with those with a partial defect. Similarly, is there a difference in patients with translocase I defect compared with glucose 6 phosphate defect? Is there a difference in liver glucose production by patients with the translocase I defect who have milder manifestations when compared with the more severely affected?		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00411-01 HGB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Evaluation of Nalmefene, an Endorphin Antagonist, in the Control of Appetite

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James B. Sidbury

Head

HGB, NICHD

## COOPERATING UNITS (if any)

Pamela Brye RD, CC, NIH

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Disorders of Carbohydrate Metabolism

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.2

## PROFESSIONAL

0.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The study was designed to assess the efficacy of nalmefene--a third generation naloxone in the control of appetite. The initial subjects were Prader-Willi Syndrome patients. The medication was increased stepwise over a three week period to 40 mg per day. The patients were then followed for two months in the outpatient department, receiving nalmefene for two weeks, alternating with placebo for two weeks. A final week in the Clinical Center was used for careful evaluation. No effect of the medication was found on food intake.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00909-07 HGB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Fetal Alcohol Syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Anil B. Mukherjee	Head HGB, NICHD
Others:	Sondra W. Levin	Medical Staff Fellow HGB, NICHD
COOPERATING UNITS (if any) M. Evans (Wayne State Univ., Detroit Michigan) B. Cowan (University Mississippi, Jackson, Mississippi) P. Martin (Vanderbilt University Med. Center, Nashville, TN)		
LAB/BRANCH Human Genetics Branch		
SECTION Section on Developmental Genetics		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p>             The role of genetic predisposing factors in developing fetal toxicity of ethanol is continued. During the past year we have studied the transketolase abnormality in cultured fibroblasts from a diabetic patient who developed Wernicke's encephalopathy when treated with tolazamide, a hypoglycemic agent. Additionally, three diabetic kindreds without any history of Wernicke's encephalopathy and four normal controls were also studied. We found that the patient with tolazamide induced Wernicke's encephalopathy and one of the three diabetic kindreds had abnormal <math>K_m</math> of transketolase for TPP similar to the ones found previously in post-alcoholic Wernicke's-Korsakoff syndrome. These data suggest that transketolase abnormality is prevalent in the population and the individuals with this abnormality may be predisposed to developing thiamine deficiency syndromes either by drugs that increase thiamine utilization or by ethanol abuse. To investigate whether or not this enzyme abnormality is associated with FAS we studied 2 cultured amniotic fluid cell lines where the neonates were diagnosed to have FAS by several criteria. Three control amniotic fluid cell lines from normal pregnancies were also investigated. Preliminary results suggest that both amniotic cell lines with FAS pregnancies have abnormal transketolase enzyme as suggested by the determination of <math>K_m</math> of this enzyme for TPP and compared to control values. Studies in progress will investigate the cells from more FAS, normal control pregnancies as well as cells derived from pregnancies where mother had a history of alcohol abuse but the baby was unaffected. In a related study we have investigated the transketolase enzyme kinetics in fibroblasts from ten members representing three generations of an Amish Family. The results show a pattern of autosomal recessive type of inheritance of the abnormality as previously speculated by McKusick. We have recruited three patients with FAS in our clinical protocol to search for a genetic factor(s) in FAS as well as to study the pathophysiology of this common disorder.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00910-07 HGB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Uteroglobin: Physiological Function and Genetic Regulation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Anil B. Mukherjee Head HGB, NICHD

Others: Lucio Miele Visiting Fellow HGB, NICHD  
 Sondra Levin Guest Researcher HGB, NICHD  
 Tadahiho Kikukawa Visiting Fellow HGB, NICHD  
 Eleonora Miele Guest Researcher HGB, NICHD

COOPERATING UNITS (if any) B. Cowan (School of Med., Univ. Mississippi); H. Zacur (Johns Hopkins Med. School, Baltimore, Md.); M. Evans (Wayne State University, Detroit, MI); P. D. Wightman (Merk Sharp & Dohme Res. Lab.); N. Dubin (Johns Hopkins Hosp. Baltimore, Md.); R. Dhanireddy (Georgetown Univ. Hosp., Wash., D.C.)

## LAB/BRANCH

Human Genetics Branch

## SECTION

Section on Developmental Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.50

## PROFESSIONAL:

2.25

## OTHER:

.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

During the past year we have shown that uteroglobin (Utg) is a potent inhibitor of phospholipaseA<sub>2</sub> (PLA<sub>2</sub>) activity in cultured cells as well as in a cell-free system. This suggested that Utg may modulate tissue prostanoids by restricting the level of arachidonate. To study this and the regulation of the Utg gene we have established SV40 ts mutant-transformed cell lines from all organs of the rabbit, known to contain Utg. Two such cell lines derived from the uterine endometrium (RBE-7 and H5DC) have so far been characterized. At 39.4°C, when stimulated with progesterone (10<sup>-9</sup>M) the transcription (as measured by dot and Northern blotting) and translation (as measured by Utg RIA) of the Utg gene occur at a rapid rate in these cells. We also found that these cells when treated with phorbol ester, a tumor promoter and an inflammatory agent, increases PLA<sub>2</sub> activity by 10-fold with significant increase in tissue prostanoids (PGF<sub>2</sub>α, PGE<sub>2</sub>, 6-KetoPGF<sub>1</sub>α and TBX<sub>2</sub>) as measured by HPLC and RIA and compared to control values. When these phorbol ester treated cells were further treated with Utg (20μM) 78% reduction in PLA<sub>2</sub> activity occurred in 12 hours and prostanoid levels decreased 85% of control values. To determine whether endogenous Utg production in these cells would reduce PLA<sub>2</sub> activity we treated these cells with progesterone (10<sup>-9</sup>M) for 24 hours when Utg production is detectable. Significant reduction in PLA<sub>2</sub> activity as well as prostanoid levels were observed in these cells. The reduction of PLA<sub>2</sub> activity and/or prostanoid level were not observed when either cyclohexamide, a protein synthesis inhibitor, or an antibody to Utg was administered following progesterone treatment. Preliminary in vivo experiments seem to confirm the in vitro results. Additionally, we have successfully subcloned in an expression vector (pKK233-3) the mature Utg coding region from a full-length cDNA containing all the pre-Utg coding sequences. A positive clone with the right orientation of the insert was used to transform two bacterial hosts which have been producing Utg. Site directed mutagenesis studies are now in progress to determine the active site in the Utg molecule involved in PLA<sub>2</sub> inhibition.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00912-07 HGB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Gene Regulation and Cellular Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Janice Y. Chou	Head HGB, NICHD
Others:	Shuichiro Watanabe	Visiting Fellow HGB, NICHD
	Yvonne Wan	Visiting Fellow HGB, NICHD
	Grace Young	Medical Staff Fellow HGB, NICHD
	Jay Joshi	Senior Staff Fellow HGB, NICHD
	Adam Sartwell	Lab. Aid HGB, NICHD
COOPERATING UNITS (if any) Drs. I. Sun and F. L. Crane (Purdue Univ., IN); Dr. G. Yeoh (Univ. of Western Australia, Australia); Dr. I. Boime (Washington Univ, MO); Dr. L. Levenbook (NIADDK); Yoomi Choe (ATCC)		
LAB/BRANCH Human Genetics Branch		
SECTION Section on Cellular Differentiation		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	4.0	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.) <p>Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. Hybridization and nuclease S1 mapping analysis demonstrated that the 20S mature <math>\alpha</math>-fetoprotein (AFP) and the 16S variant AFP mRNAs differ in sequences at the 5' end. The first six exons of the 20S mRNA were missing in the 16S RNA.</p> <p>We demonstrated that glucocorticoid hormone promotes maturation of fetal hepatocytes in vitro. Administration of glucocorticoid to differentiated fetal hepatocytes inhibited the production of fetal protein, AFP but induced the synthesis of tyrosine aminotransferase (TAT), an enzyme which appears only post-natally. Therefore, fetal cells matured into adult hepatocytes in the presence of glucocorticoid.</p> <p>The ts adult hepatocyte line expressed a large number of liver-specific functions. In addition to synthesizing AFP, albumin, and transferrin, these cells expressed genes encoding TAT, phosphoenolpyruvate carboxykinase (PEPCK), and fibrinogen. Furthermore, we showed that the expression of these genes was ts and glucocorticoid dependent.</p> <p>Genes encoding placental alkaline phosphatase (PAP) subunits were cloned. We found both choriocarcinoma and HeLa cells expressed different-sized PAP mRNA. Prednisolone and sodium butyrate which increased PAP biosynthesis in HeLa cells induced the expression of mature PAP mRNA.</p> <p>We cloned genes encoding pregnancy-specific <math>\beta</math>-1-glycoprotein (PS<math>\beta</math>G). These probes are used to examine molecular mechanism regulating PS<math>\beta</math>G synthesis in placental cells and fibroblasts.</p>		



LABORATORY OF COMPARATIVE ETHOLOGY

- Z01 HD 00054-12     Structural and Behavioral Analysis of Vocal Communication  
                              in Squirrel Monkeys  
                              M. Biben, Ph.D.
- Z01 HD 00062-10     Brain Mechanisms of Vocal Production in Squirrel Monkeys  
                              J. D. Newman, Ph.D.
- Z01 HD 00702-06     Genetics of Primate Vocal Behavior  
                              J. D. Newman, Ph.D.
- Z01 HD 01102-05     Behavioral Correlates of Endocrine Disorders in Children  
                              Robert P. Klein, Ph.D.
- Z01 HD 01104-04     An Observational Study of Parent-Infant Interaction  
                              in a Family Context  
                              Frank A. Pederson, Ph.D.
- Z01 HD 01106-03     Developmental Continuity of Individual Differences  
                              in Rhesus Monkey Reactivity  
                              Stephen J. Suomi, Ph.D.
- Z01 HD 01107-03     Adaptation of Laboratory Reared Monkeys to Field  
                              Environments  
                              Stephen J. Suomi, Ph.D.
- Z01 HD 01108-02     Comparative Studies of Play Behavior  
                              Maxeen Biben, Ph.D.
- Z01 HD 01110-01     Intuitive Parenting of Infants in Comparative Perspectives  
                              Stephen J. Suomi, Ph.D.
- Z01 HD 01111-01     Factors Affecting Nurturant Behavior Toward Infants  
                              Frank A. Pedersen, Ph.D.



Laboratory of Comparative Ethology

- The Laboratory of Comparative Ethology (LCE) carries out a program of research directed toward the study of behavioral and biological development in humans and in nonhuman primate subjects. The influences on developmental processes of both genetic and environmental factors, and their interactions, are explored in a comparative mammalian approach in order to determine the origins and evolution of various behavioral phenotypes. Longitudinal designs are employed in most developmental studies within the laboratory, in order to address issues of ontogenic continuity vs. change, and both behavioral and physiological measures reflecting multiple levels of analysis are collected concomitantly in these studies. A major emphasis is placed on characterizing and understanding normative patterns of biobehavioral development so that deviant patterns can then be readily recognized and their consequences evaluated with respect to established norms. Experimental results in nonhuman primates are correlated with the results of longitudinal studies of human infants and their families as well as results obtained by various neuroscience techniques.

The LCE consists of 3 sections. The Comparative Behavioral Genetics section, headed by Dr. Suomi, investigates various processes underlying biological and behavioral development in rhesus monkey subjects by focusing on interactions between genetic and environmental factors that affect the course of an individual's ontogeny. Parallel patterns are then examined in human subjects. Within the Section, the Unit on Neuroethology, headed by Dr. Newman, uses neuroscience techniques to study brain mechanisms involved in the production of various types of vocalizations by squirrel monkeys and to examine subtle accoustical differences in characteristic calls between closely related New World primate species. The Brain, Behavior and Communication Section, headed by Dr. Symmes, studies the use of vocal signals by group-living squirrel monkeys, both in terms of the accoustical properties of the signals and their information content for group members. The Child and Family Research Section, headed by Dr. Pedersen, examines cognitive, emotional, and psychosocial development in human infants and children, with special emphasis on determining the effects of early family experience on psychological development. Summaries of individual research projects from each LCE section follow.

Comparative Behavioral Genetics Section

This past year a major construction project involving the complete renovation of Building 112 into a modern primate facility compatible with AALAC standards was begun and should be completed by November, 1986. Additional construction was carried out expanding the shelter inside of the 5-acre field enclosure at the NIHAC. Despite all of this construction two major longitudinal studies investigating the interaction of genetic and environmental factors in shaping individual rhesus monkey development were completed this year. In the first study, replicating an experiment performed last year, rhesus monkey infants selectively bred to be highly responsive to mild environmental challenges were cross-fostered to multiparous females who differed with respect to their own reactivity to challenge and in their characteristic maternal "style" (nurturant vs. mildly punitive)

as displayed toward previous offspring. The highly reactive infants were compared with control infants cross-fostered to similar multiparous females. Neonatal tests of reflex development, orienting capabilities, and temperament administered to these subjects during their first month of life revealed that the infants with high reactive pedigrees showed poorer orienting responses, were less mature motorically, displayed greater behavioral inhibition, and had lower predominant activity states than did control infants. Differences in these infant scores could not be attributed to either the reactivity or the characteristic maternal style of the foster mothers. The infants remained with their foster mothers throughout their first 6 months of life, and during this time there were few apparent differences between high reactive and control infants on standard measures of behavioral development. Individual differences among infants during this period were best predicted by the maternal style of their foster mothers, in that infants of punitive foster mothers showed less ventral contact with their foster mothers, locomoted and explored their environment more, and displayed higher levels of self-directed behavior than did infants with nurturant foster mothers; these differences became greater as the infants passed through the period of weaning. When the subjects were 6 months old, they were briefly separated from their foster mothers. During the periods of separation dramatic differences in response emerged between subjects of high reactive pedigree and their controls, independent of foster mother reactivity or maternal style, with high reactive monkeys displaying higher levels of self-directed behavior, less locomotion and exploration (coping), greater passivity, and higher increases over preseparation levels of plasma cortisol. When the subjects, now juveniles, were reunited with their foster mothers, differences between high reactive subjects and controls again disappeared. During reunion periods the best predictor of differences between subjects was the relative reactivity of the foster mothers, in that juveniles with high reactive foster mothers had higher levels of ventral contact, locomoted and explored less, and displayed higher levels of disturbance behavior than did those with moderately reactive foster mothers. These differences emerged independent of subject reactivity or foster mother maternal style. Subjects were permanently moved into groups containing agemates at 8 months of age and have remained in these peer groups to the present. Within these now stable groups the high reactive juveniles were initially more aggressive than were controls, and they continued to show higher levels of clinging social contact and were less passive than controls during the next 6 months. Those high reacting subjects who had been reared by nurturant foster mothers rose to the top of the group's dominance hierarchy. These monkeys will continue to be studied at least to early adulthood, but the findings to date clearly indicate that individual differences displayed during early development are the product of both genetic and environmental factors and that to the degree that these factors can be specified, rather precise predictions of developmental outcomes in various settings are possible.

Genetic-environmental interactions were further investigated in a second longitudinal study comparing nursery-reared vs. mother-reared rhesus monkeys on both neonatal reflex, orienting, and temperament assessments and performance on tests of cognitive capabilities as juveniles. Although the two groups differed significantly on several of the neonatal measures, their mean scores on the cognitive tests, administered at 8 months of age, did not differ significantly. However, the profiles of neonatal scores associated with optimal performance on the later cognitive tests were dramatically different for the mother-reared and nursery-reared subjects, in that mother-reared juveniles who as neonates had "easy" temperaments and low arousal levels had the best performance within their



rearing group on the cognitive tests, whereas those nursery-reared subjects who were "fussy," highly aroused, and uninhibited behaviorally when assessed as neonates had the best 8-month performances within their rearing group. Thus, the same top scores on the cognitive test battery were achieved by individuals with very different neonatal temperaments when they grew up in different rearing circumstances.

During the past year a number of new measures representing different levels of analysis were incorporated into ongoing longitudinal studies of rhesus monkeys differing in their behavioral phenotypes. A means for recording heartrate and monitoring vagal tone (a measure of sympathetic/parasympathetic balance) in free-ranging monkeys via a miniaturized telemetry system was perfected and implemented in a study comparing the responses of juvenile rhesus monkeys differing in genetic pedigree for reactivity to placement with an unfamiliar agemate in a laboratory playroom filled with toys for a 1-hour period, a paradigm adapted from research on human preschool children with different temperaments. In this situation it was found that high reactive juveniles had higher and more stable heartrates upon initial entry into the playroom and showed less decline in heartrate over the 1-hour session than did control subjects. The vagal tone measures are currently being analyzed.

Several measures of immune system response were assessed in other longitudinal studies of rhesus monkey development. In one study, levels of the immunoglobulins IgG and IgM were monitored over the first 6 months of life in nursery-reared, mother-reared, and foster mother-reared rhesus monkeys. IgG levels on the first day of life were equivalent to adult levels and thereafter declined steadily over the first month of life in all 3 rearing groups, suggesting that, as is the case for human infants, IgG is transferred to the fetus through the placenta but is not augmented postnatally via mother's milk. Major individual differences in levels of IgG appeared in succeeding months, with some subjects showing a continuing decline and others showing a rebound after the third month. IgM values showed a different developmental trend with sharp increases from birth to day 15, followed by a moderate decline to day 30 and relative stability thereafter, perhaps reflecting a primary antibody response to the initial bacterial colonization of the gut, again similar to the case in human infants. At 6 months of age the nursery-reared subjects were briefly separated from their peer partners, with IgG and IgM sampled immediately before and following the separations. Small but statistically significant decreases in IgG, but not in IgM, were associated with the separation manipulation. In addition, individual differences in both IgG and IgM were remarkably consistent not only throughout the subjects' first 6 months but also following the brief separation. These findings suggest that immunoglobulin levels might serve as useful markers of relative vulnerability to social challenges and in some individuals might indicate increased risk in morbidity following such challenges.

A different type of immune system measure, response to mitogen challenge, was employed in a study investigating the establishment of dominance hierarchies in newly formed groups of rhesus monkey juveniles. Both mother-reared and nursery-reared subjects who had lived in the same groups during their first year of life, but who then had been moved to different social groups during their second year, were returned to their original social groupings at 2 1/2 years of age. Blood samples were obtained from each subject just prior to, 1 1/2 hours after, and 4 days after re-constitution of its original social group. In addition to assays

for plasma cortisol, ACTH, and CRF, whole blood sampled from each subject was cultured and subsequently exposed to 2 different mitogens, concanabin A and phytohemagglutinin (PHA) in Dr. Tamarkin's laboratory (CPSB, NIMH). Results analyzed to date indicated that (a) subjects placed back in their original social groups rapidly established a dominance hierarchy that was virtually identical to the hierarchy in those original groups, despite the intervening 1 1/2 year period of differential social experience, (b) there were marked and rapid changes in response to mitogen challenge, with significant declines for both types of mitogen within 1 1/2 hours of group formation, followed by a general rebound 4 days later, and (c) there were marked and consistent individual differences in response to mitogen challenge, with the highest ranking subjects showing the greatest responsiveness at each sampling point, especially right after group formation. Analyses of the plasma cortisol, ACTH, and CRF levels, as well as correlation of specific behavioral measures with both mitogen challenge results and HPA activity are currently in progress.

Data from a study of the behavioral and physiological effects of imipramine treatment of adolescent rhesus monkeys under conditions of group vs. individual housing were subjected to additional analyses this past year. Most notably, analysis of blood levels of imipramine and its metabolites in each subject revealed marked differences in imipramine turnover when subjects were housed together in familiar social groups, as opposed to being individually housed for 4-day periods. In particular, blood levels of imipramine were much lower, and levels of the metabolites OH-imipramine, desimipramine, and OH-desimipramine were much higher, when subjects were individually housed. These findings suggest different pathways for metabolizing imipramine under conditions of environmental challenge. A replication study, involving acute rather than chronic administration of imipramine, has been completed and the data are currently under analysis.

An ongoing study of behavioral development and social organization was continued in a group of 13 year-old rhesus monkeys who despite being reared without mothers and with only minimal peer contact have displayed species-normative behavioral repertoires (as have 2 generations of progeny) since being moved to an outdoor enclosure. As was previously mentioned, there was an interruption in the study due to construction involving major modifications of the monkey shelter within the 5-acre enclosure, restricting observational data collection for a 5-month period. Nevertheless, those data that were collected continued to verify the species-normative behavioral patterns in the adults and ontogenic changes in the infants, the characteristic social organization of the troop along matriarchal lines, the species-appropriate changes in social rank among troop members, most notably the drop in dominance and eventual peripheralization of adolescent males, and the successful maternal care displayed by second-generation females. In addition, another infant born outside the group was successfully cross-fostered to one of the founder females who had just delivered a still-born infant, and a study investigating the manipulative skills of transmission of tool-use-like behavior as a function of age and matriarchal lineage was initiated. Plans for a second and third enclosure progressed, with data collection initiated on some of the monkeys who will be released into the new enclosures.

The Unit on Neuroethology, headed by Dr. Newman, completed several studies investigating brain mechanisms involved in the production of specific vocal patterns in squirrel monkeys. The role of alpha-adrenergic receptors in production of isolation calls was examined by comparing the effects of different doses of the alpha



antagonist clonidine, the alpha-2 antagonist yohimbine, and the alpha-1 antagonist prazosin. Call rate decreased in dose-dependent fashion with clonidine administration and increased with increasing doses of yohimbine. When a moderate dose of yohimbine was given concomitantly with the highest dose of clonidine, the suppressive effects of clonidine were reversed, but when prazosin, the alpha-1 receptor antagonist, was given concomitant with clonidine, there was no reversal of call suppression, thus implicating alpha-2, but not alpha-1 receptors in isolation call production. The antidepressant imipramine also suppressed production of isolation call in these subjects. Another series of studies examined the effects of anticholinergic compounds on production of both isolation and alarm calls. Benactyzine increased alarm call production while decreasing the rate of isolation calling. These benactyzine effects could be blocked by pretreatment with the cholinergic physostigmine (which crosses the blood-brain barrier) but not with the cholinergic neostigmine (which does not cross the blood-brain barrier).

This past year the Unit also completed a long-term study of inheritance patterns of species-typical isolation calls in hybrid offspring of cross-species breeding between sympatric populations of 2 squirrel monkey species and between allopatric populations of the same species (Gothic-arch vs. Roman-arch squirrel monkeys). Isolation calls of the hybrids resulting from sympatric crosses most closely resembled the characteristic Roman-arch phenotype, while calls of allopatric hybrids more closely resembled the Gothic-arch phenotype. A second long-term comparative study of isolation calls among members of the prosimian genus Lemur was also completed this year with the recording of calls from Lemur mongoz infants born at the Duke University Prosimian Primate Center. Comparative analyses of sound spectrographs revealed an increasing amount of broad-frequency noise overriding the basic tonal structure of isolation calls from Lemur mongoz, Lemur fulvus, Lemur macaco, Lemur coronatus, and Lemur catta, respectively. An additional study of vocal patterns in New World and prosimian primate species that routinely produce twin births was initiated with the recording of isolation calls from Cheirogaleus medius, Varrecia variegata, and Lemur fulvus infants (Lemuridae family), and from Callithrix jacchus and Cebuella pygmae infants (Callitrichidae family).

Finally, this year two major research projects involving developmental study of human infants were initiated in the Comparative Behavioral Genetics Section. A pilot study of individual differences in behavioral and physiological responsiveness to mild environmental challenge was conducted in collaboration with Dr. K. Grossmann (University of Regensburg, West Germany) and Dr. S. Porges (University of Maryland). Ten-month-old infants were brought into an examining room by their mothers and after producing a saliva sample (to be assayed for saliva cortisol) and being fitted with a nonobtrusive telemetry system for recording heartrate and vagal tone data, the infants were run through a standard pediatric examination, after which a second saliva sample was obtained. Two months later each infant returned to the laboratory with his or her mother and after producing a saliva sample and being fitted with the heartrate telemetry system again, the infant was run through the Ainsworth Strange Situation Procedure (a standard experimental paradigm in the human mother-infant attachment research field), after which a second saliva sample was obtained. Both experimental sessions for each infant were videotaped for subsequent behavior coding. The data from this study, currently under analyses, will delineate the effects of the two manipulations on saliva cortisol, heartrate, vagal tone, and behavior, permitting assessment of the stability of individual differences on each of the measures across the two

situations. These findings can then be compared with data from parallel studies of individual differences in response to environmental challenge in rhesus monkey infants and juveniles, in which considerable stability over time and across situations has already been found.

The second set of human infants studies were carried out in collaboration with Drs. H. and M. Papousek of the Max Planck Institute of Psychiatry in Munich and with Dr. Symmes, Chief of the Brain, Behavior, and Communication Section of the LCE. These studies focused on the phenomenon of "intuitive parenting," in which preverbal human infants elicit a distinctive pattern of communication from parents and other caretakers that includes alteration in the form of speech ("baby-talk"), exaggerated facial expressions, and maintenance of eye-to-eye contact at a fixed distance; such distinctive communicative patterns are produced by parents automatically and usually without conscious awareness that they are interacting with the infant in different fashion than they would in conversations with adults. In the main study for which data collection was completed this year, mothers whose native language was either English or Mandarin Chinese (a language characterized by extreme tonality) brought their 2-month-old infants into a laboratory playroom, and in that setting their normal interactions with their infants were videotaped over a half-hour period. When their infants were 4 months old the mothers and their infants returned to the laboratory playroom for a second interaction session, which was also videotaped. The pitch patterns and contours of both mothers and infants were then subjected to microanalytic spectrographic analysis using computer-aided array processing techniques. The maternal utterances have also been transcribed, translated (for Chinese speaking mothers), and coded for linguistic content, and microanalysis of changes in both the mothers' and infants' facial expression have begun. Additional cross-cultural comparisons with existing videotapes of mother-infant interactions taken from West German, African pigmy, Yucatan Indian, and American deaf samples are also underway, with preliminary data indicating great generality of the phenomenon of intuitive parenting across all cultures sample, but with some differences in the relative frequency and developmental course of the phenomenon from one culture to another. Finally, examination of tapes of early mother-infant interactions in pigmy chimpanzees and lowland gorillas, two of Homo sapiens closest genetic primate relatives, indicate some evidence for intuitive parent-like communication in the tactile mode but a absence of the pattern of vocal and eye-to-eye visual exchange so characteristic of intuitive parenting in every human culture studied to date.

#### Brain, Behavior, and Communication Section

During the past year the technical capabilities of the Section on Brain, Behavior, and Communication (SBBC) were greatly expanded when the Section Chief, Dr. Symmes, acquired and installed a new digital sound analysis system based on a DEC 11/73 computer equiped with color graphics. This system now permits direct analysis of monkey calls at frequency ranges well beyond human hearing and effects enormous savings in operator time in processing data samples. This system has been effectively used to process audio tapes and to develop digital records suitable for sophisticated statistical analysis of the frequency domain description obtained in human mother-infant interaction, as described in the previous section, and in several analyses of monkey data. With recently acquired Kay digital sound spectrographic apparatus, with which the computer is interfaced, the SBBC laboratory is now fully updated and in fact has capabilities to analyze biological sounds second to none.



Employing this new equipment, Drs. Symmes and Biben have collaborated on several projects studying various elements of squirrel monkey communication, with the result that several valuable new steps have been taken in the study of squirrel monkey vocal signalling as a model language. Although the resemblance between any nonhuman primate vocal system and human language is distant, new developments in this field of study, and in this laboratory in particular, reduce the distance and provide insights about those linguistic properties which are primitive and general. For example, the quiet exchange of "chuck" calls between female squirrel monkeys with an established bond of friendship appears to be an excellent model of "conversations," i.e., vocal exchanges where the structure and information content of later utterances are modified by variables present in earlier vocalizing. Expanding on previous findings collected from Gothic-arch squirrel monkeys, these researchers have collected new data on chuck calls from Roman-arch subjects. This type of Saimiri is generally considered a subspecies, and among the best described phenotypical characteristics which distinguish them as a subspecies are vocal signals. Previous studies of subspecific differences were not extended to affiliative contexts, as in the present project. The new data suggest more similarities than differences between the subspecies' use of chucks, along with a few structural distinctions. One exception is the existence of a more rigid temporal "rule" regarding spacing of calls in Roman-arch animals. Affiliative partners respond very promptly or not at all to "question" chucks. It is possible that such temporal spacing contributes to recognition of familiar animals.

In the first of two new studies on the ontogeny of vocal communication in squirrel monkeys, it was found that cackle calls (used at other times and by adults) are given by the dominant partner in play bouts among subadult peers, and play peep calls (used only by young animals when playing) are given by the subordinate partner. When role reversal occurs, as it does frequently in play between similar age males, play peeps are given by both players. Thus usage corresponds with social relationship, as it does in the quiet affiliative context. The rate of play peeping and the structural complexity of the calls correlate with and in fact predict the duration of play bouts. The conclusion was drawn that play peeps reflect motivation to play rather than serving as signals about the content of play or as metacommunicative reassurance to partners. The latter view, focusing on a hypothesized need to avoid a progression from play to fighting, has had some acceptance as speculation in the previous literature and is clearly inconsistent with these results, which are the first systematic study of playful vocalizations in a nonhuman primate.

A second ontogenic study nearing completion has addressed the question of early mother-infant vocal interactions, also in squirrel monkeys. It is widely believed that monkey vocalizations are preprogrammed and develop normally without a learning process. This belief has some support from description of acoustic structure, but extension of the conclusion to functional use goes far beyond available evidence. The present initiative is intended to describe in some detail the nature of early vocal experience and usage and to follow functional development for several years. The study has already disclosed the preeminence in the first months of a call type not previously described, although probably heard by field observers. The "coax" call is used by mothers and juvenile females and results in the infant returning to the mother or aunt and, at times, to cause the infant to assume the nursing position. Pitch contours of this tonal call are being analyzed in terms of context, and they provide an unusual opportunity for comparison with similar data at the human level, as described in the previous section.

## Child and Family Research Section

The Child and Family Research Section, headed by Dr. Pedersen, continued to work on two long-term projects this past year and initiated three new studies investigating the development of nurturant responses by parents toward infants. The first longterm project has involved the observational study of parent-infant interactions in a family context. One focus of the project has been on the effects of different types of short-term maternal separations on infant socio-emotional development. In families in which mothers began employment prior to their infants' third month of life, those infants judged to have secure vs. insecure maternal attachment relationships at 15 months did not differ on any of 4 measures of amount of employment-related separation they had experienced. However, a composite measure of continuity of substitute care was strongly related to secure/insecure attachment. Infants with a secure maternal attachment were equally distributed across the full range on the continuity scale. In contrast, 86% of infants with an insecure attachment experienced substitute care characterized by lack of high continuity. These findings are the first to link factors in substitute care environments to the mother-infant attachment relationship. The question being addressed in ongoing research is whether lack of continuity in substitute care exerts a direct effect on the infant's attachment relationship or whether this factor is a marker of a more basic characteristic of the mother, such as her sensitivity.

In studies of the father's role in the infancy period, follow-up observations of father-infant interaction at age 1 year for men who evidenced contrasting affective reactions during the early months of parenthood. Men who reported in interviews that they had experienced periods of "blues" or dysphoric mood, as contrasted to men who did not report such feeling states, were found in home observations at 3 months to have a more disengaged style of relating to their babies. They spent more time physically remote from the baby, touched their babies less frequently, and they provided less frequent caregiving and affection than did the comparison group. At age 12 months, statistically significant interactions of group by age were found on these measures. The nature of the interactions was that the areas of behavior that were sensitive to dysphoric mood and were engaged in at rates lower than the comparison group at 3 months were, at 12 months, engaged in more actively than in the comparison group. In addition, a factor that was associated with dysphoric mood at 3 months, problems in the marital relationship, was reported by the men and their wives as significantly improved at age 12 months. These findings provide evidence of a compensatory process at work in men who had initial adaptational difficulties. The pattern to the findings is consistent with a transactional model of early experience that emphasizes the "self-righting" potential in human adaptations.

The second long-term project, headed by Dr. Klein, examined the nature and relative incidence of behavior problems in samples of children receiving hormonal treatments for precocious puberty. Analyses completed this year focused on describing the combined influence on adjustment of age of onset, duration of symptoms before onset of treatment, and pretreatment medical status as indicated by bone age, vaginal bleeding, relative height, Tanner stage, and, for the idiopathic precocious puberty group, the levels of gonadotropins and sex steroids. Both age of onset and delay between symptom onset and beginning treatment appeared equally important for adjustment. Presence of vaginal bleeding showed no relationship whatsoever to adjustment. Extensive multiple correlational analyses

were carried out to examine the combined effect of the various medical status indicators. In general, these factors had a synergistic effect, i.e., their influence was stronger when taking into account the other factors than when looking at their influence individually. One example of this is Lutenizing Hormone (LH). Looked at individually, levels of LH had a slight positive influence on adjustment; looked at in conjunction with other biomedical variables, relative levels of LH had a negative influence. Another finding disclosed during this year's analyses was that that estradiol levels were significantly related to adjustment, but none of estradiol's putative physiological effects (i.e., bone age, relative height, breast stage, and vaginal bleeding) significantly covaried with the adjustment measure.

Three new studies initiated this past year examined the development of parental behavior, especially that characterized as nurturant, toward infants. The first study addressed the intergenerational transmission of nurturant roles for females and males. The general hypothesis being tested was that beginning very early in their children's lives, parents communicate differential expectations in their play behavior with male and female children regarding care for babies, with mothers fostering stronger nurturant expectations than fathers, who in turn differentiate their role expectations for males and females more strong than mothers. The second study involved an intervention during the pregnancy period for first-time expectant mothers. The intervention procedure involved having the expectant mother (a) handle a young infant on three different occasions, (b) observe her behavior with the infant on videotape, and (c) receive feedback about her behavior, was hypothesized to reduce anxiety, heighten the mother's sensitivity to different arousal states in the young infant, and facilitate her making appropriate responses to behavior emitted by the infant. The third study compared two groups of expectant parents who differ in exposure to a specific psychological stress, the emotional sequelae of a previous pregnancy loss, in order to determine whether the loss (miscarriage, stillbirth, or neonatal death) contributed toward anxiety, depression, and a dysfunctional parental adaptation that could interfere with nurturant behavior toward the young. For each study, pilot investigations have been completed during the year and formal data collection is currently underway.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00054-12 LCE

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and behavioral analysis of vocal communication in squirrel monkeys

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. Symmes Head LCE, NICHD

Other: M. Biben Senior Staff Fellow LCE, NICHD  
N. Masataka Visiting Fellow (through 1/86) LCE, NICHD  
D. Bernhards Bio. Lab. Technician LCE, NICHD  
G. Hetzel Animal Caretaker LCE, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Section on Brain, Behavior, and Communication

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.4

## PROFESSIONAL:

1.5

## OTHER:

0.9

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

We have continued the study of squirrel monkey calls used in social contexts characterized by quiet affiliative behaviors and lack of internal aggression or external threat. Our program of research on this model of "conversation" in infrahuman primates has yielded significant findings regarding both structural and temporal rules governing vocal exchanges. We have devoted much of our time this year to statistical analysis and publication of these findings.

We have set a new goal of describing in some detail the development of vocal usage in young squirrel monkeys raised normally in social groups. The significance of this program objective lies in identification of the maturational parameters of social vocalizations (as opposed to separation induced calls such as the Isolation Peep, which we have described in prior work) and of possible influences of learning on the structure and use of these social vocalizations. Early work has identified a call used in very early exchanges between mothers or other adult females and infants, which has been poorly described previously and not characterized acoustically.

We have begun late in FY86 a collaboration with Drs. Hanus and Mechthild Papousek within the LCE involving the quantification of acoustic structure in vocal interactions between human mothers and their preverbal infants. The recently-acquired BBCS computer facilities have proved helpful in analyzing the substantial database of tape recorded material collected by these investigators and their collaborators. Specifically, pitch contours of several thousand utterances occurring in different behavioral contexts have been obtained and stored as digital records. Methods we have employed in the past with tonal vocalizations of monkeys are being applied here to combine, average, and extract descriptors of vocalizations across subjects and contexts. A fuller description of the methods and objectives of this project appears elsewhere.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00062-10 LCE
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Mechanisms of Vocal Production in Squirrel Monkeys		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  PI: J. D. Newman      Research Physiologist      LCE, NICHD  Other: D. Bernards      Bio. Lab. Technician      LCE, NICHD G. Hetzel      Animal Caretaker      LCE, NICHD		
COOPERATING UNITS (if any)  LCS, NIMH (P. D. MacLean); CNB, NIMH (J. R. Glowa); Johns Hopkins School of Medicine (J. C. Harris)		
LAB/BRANCH Laboratory of Comparative Ethology		
SECTION Comparative Behavioral Genetics		
INSTITUTE AND LOCATION NICHD, NIH		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.2	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided )  This project investigates the effect of neurological and pharmacological interventions on the incidence and structure of squirrel monkey vocalizations emitted during standardized conditions in the laboratory. A collaborative study with P. MacLean has focused on the effect of various cerebral lesions on the production of the isolation call (IC). We tested whether ablation of midline frontal neocortex lying peripheral to a previously discovered critical band of limbic cortex would interfere with IC production. We found that in the absence of this neocortical tissue there was full recovery of spontaneously produced ICs. Other current work is directed at the chemical substrates mediating vocal production. The role of the adrenergic system in IC production was studied with J. Harris. We established dose-response relationships for the alpha-adrenergic agonist clonidine and alpha-2 antagonist yohimbine, using rate of IC production as the behavioral response measure. Both drugs exhibit dose-dependent effects on IC production, the agonist decreasing and the antagonist increasing calling rate. Since yohimbine reverses the clonidine-induced reduction of IC calling rate, we tested the specificity of an alpha-2 mechanism in mediating this behavior by substituting the alpha-1 antagonist prazosin for yohimbine. We found that this drug, in a dose of 0.25 mg/kg, was completely ineffective in reversing clonidine-induced vocal suppression. In another experiment, we established that adrenergic and opiate mechanisms can interact in mediating IC calling rate. Systemic administration of yohimbine concurrently with naloxone resulted in up to 7-fold increases in calling over rates following treatment with either drug alone. In a study with J. Glowa, the role of cholinergic mechanisms in mediating alarm call production was investigated. We found that a centrally active cholinomimetic, physostigmine, was ineffective by itself, but it blocked the increased calling produced by the anticholinergic compound benactyzine. Cholinergic or anticholinergic compounds tested were without effect on IC production.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00702-06 LCE

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Primate Communication

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. D. Newman

Research Physiologist LCE, NICHD

## COOPERATING UNITS (if any)

LCS, NIMH (M.Z. Wamboldt); Duke University Primate Center

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Comparative Behavioral Genetics

## INSTITUTE AND LOCATION

NICHD, NIH

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL

0.6

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project investigates the role of genetic factors in primate vocal development. Research strategies employed in these studies include analysis of vocal traits in hybrid offspring having vocally distinctive parents, comparing the vocal traits of full and half sibs with unrelated age-matched infants, and analysis of the effects of differential rearing on normal vocal development. In FY 86, a long-term project studying the inheritance patterns of the species-typical isolation call (Isolation Peep; IP) in hybrids of crosses involving vocally distinctive sympatric populations (Peruvian Gothic x Peruvian Roman) was completed. Hybrids studied until 2 years of age produce IPs with acoustic parameters that are intermediate to IPs from the parents, but whose overall structure more closely resembles that of the Roman parent. A comparative study of the isolation calls of prosimian primates (family Lemuridae) was also completed, following the successful recording of the isolation calls from a second Lemur mongoz infant, along with representative samples from both parents. The data from this study show that acoustic parameters differentiating the isolation calls of different lemur species are present in the newborn infant and persist into adulthood. Adult members of the genus Lemur engage in vocal dialogues with their separated offspring, using adult versions of the infant's isolation call. A study begun this year is focused on examining the vocal characteristics of primate twins, with the goal of developing a primate model for the study of shared vocal traits in twins. Five species of prosimians and two species of marmosets-- all having a high incidence of multiple births-- were studied, and the species-specific attributes of their isolation calls determined. The infants of 2 nocturnal prosimians (mouse lemur, Microcebus murinus; dwarf lemur, Cheirogaleus medius) produce isolation calls in the 18-20 kHz range and are more vocal during their active cycle in a test environment illuminated with long wave length light.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01102-05 LCE
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Behavioral Correlates of Endocrine Disorders in Children		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:        R. P. Klein                      Senior Research Investigator        LCE, NICHD  Other:    N. F. Gist                      Research Psychologist                LCE, NICHD M. Fivel                        Research Pscyhologist                LCE, NICHD		
COOPERATING UNITS (if any) Developmental Endocrinology Branch, NICHD; Laboratory of Developmental Psychology, NIMH; Child Studies Center, University of Maryland; Division of Endocrinology, Children's Hospital Medical Center; University of Minnesota Medical School (Sonis)		
LAB/BRANCH Laboratory of Comparative Ethology		
SECTION Child and Family Research Section		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 0.8	OTHER: 1.7
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)  This project encompasses a series of studies examining the behavioral correlates of endocrine disorders in young patients, including children with precocious puberty, Turner's syndrome, and growth hormone deficiency. A first objective was to determine whether these children are at risk for problems in psychosocial adjustment. In a sample of children with precocious puberty, we reported that these children do, in fact, show an above-normal incidence of a variety of adjustment problems. A current objective is to ascertain the factor(s) responsible for this finding. Analyses during the past fiscal year have focused on the combined influence of age of onset, duration of symptoms before treatment was begun, and pretreatment medical status as indicated by bone age, relative height, pubic hair stage and the levels of gonadotropins and sex steroids. In general these factors had a synergistic effect, i.e., their influence was stronger when taking into account the other factors than when looking at their influence individually.		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01104-04 LCE

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

An Observational Study of Parent-Infant Interaction in a Family Context

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	F. A. Pedersen	Head	LCE, NICHD
Other:	R. L. Cain	Research Psychologist	LCE, NICHD
	J. D. Suwalsky	Research Psychologist	LCE, NICHD
	V. Smeriglio	Guest Researcher	LCE, NICHD

## COOPERATING UNITS (if any)

Department of Maternal and Child Health, School of Hygiene and Public Health,  
Johns Hopkins University

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Child and Family Research Section

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.1

## PROFESSIONAL:

1.6

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- |  |  |                                      |
|--|--|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input checked="" type="checkbox"/> (a1) Minors        |  |                                      |
| <input checked="" type="checkbox"/> (a2) Interviews    |  |                                      |

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This project currently encompasses four areas of investigation based on several different samples with a total of approximately 200 families as participants. All studies were conducted with middle class families and first-born infants. The focal period of current research is from early infancy through the first two and a half years of life. Procedures vary with each sample, but include observations of mother-infant and father-infant interaction in the natural home environment, structured interactions in the laboratory, interviews, and questionnaires. The first area of inquiry concerns the effects of maternal workforce participation on the child's early experiences. Structured laboratory observations of mother-toddler play were conducted to test hypotheses related to previous findings that when negative consequences have been reported for children of employed mothers, these tend to occur for males. A second inquiry focused upon naturally occurring mother-infant separation experiences. In a sample of employed mothers, amount of mother-infant separation was not related to a secure/insecure attachment. Continuity of substitute care during separations, however, was related to quality of attachment. A third area of inquiry is concerned with the father's role in the family. Follow-up observations of father-infant interaction at 1 year of age were analyzed for two groups of men who at 3-months evidenced contrasting affective reactions to parenthood and had distinctive patterns of involvement with their infants. The fourth area of inquiry concerns 3-person interactions, the mutual regulation of visual, vocal, proximity, and contact behavior of mothers, fathers, and infants. Patterns of behavior are being examined that vary depending upon the parents' psychological accessibility to the child and their degree of verbal engagement with one another.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 01106-03 LCE																				
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986																						
<b>TITLE OF PROJECT</b> (80 characters or less Title must fit on one line between the borders ) Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity																						
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I.:</td> <td style="width: 35%;">S. J. Suomi</td> <td style="width: 30%;">Head</td> <td style="width: 20%;">LCE, NICHD</td> </tr> <tr> <td>Other:</td> <td>C. E. Eisele</td> <td>Research Psychologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>J. M. Scanlan</td> <td>Research Psychologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>M. Champoux</td> <td>Research Psychologist</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>K. M. Grossmann</td> <td>Fogarty Visiting Fellow</td> <td>LCE, NICHD</td> </tr> </table>			P.I.:	S. J. Suomi	Head	LCE, NICHD	Other:	C. E. Eisele	Research Psychologist	LCE, NICHD		J. M. Scanlan	Research Psychologist	LCE, NICHD		M. Champoux	Research Psychologist	LCE, NICHD		K. M. Grossmann	Fogarty Visiting Fellow	LCE, NICHD
P.I.:	S. J. Suomi	Head	LCE, NICHD																			
Other:	C. E. Eisele	Research Psychologist	LCE, NICHD																			
	J. M. Scanlan	Research Psychologist	LCE, NICHD																			
	M. Champoux	Research Psychologist	LCE, NICHD																			
	K. M. Grossmann	Fogarty Visiting Fellow	LCE, NICHD																			
<b>COOPERATING UNITS</b> (if any) LCS, NIAAA (M. Linoilla, D. Higley); CNB, NIMH (T.R. Insel, R.D. Delizio); LN NIMH (E. Murray); Primate Laboratory, University of Wisconsin-Madison (C. Coe, M.L. Schneider), Department of Psychology, University of Regensburg (K. L. Grossmann)																						
<b>LAB/BRANCH</b> Laboratory of Comparative Ethology																						
<b>SECTION</b> Comparative Behavioral Genetics																						
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892																						
<b>TOTAL MAN-YEARS</b> <div style="text-align: center;">5.3</div>	<b>PROFESSIONAL:</b> <div style="text-align: center;">3.0</div>	<b>OTHER:</b> <div style="text-align: center;">2.3</div>																				
<b>CHECK APPROPRIATE BOX(ES)</b> <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews													
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither																				
<input type="checkbox"/> (a1) Minors																						
<input type="checkbox"/> (a2) Interviews																						
<b>SUMMARY OF WORK</b> (Use standard unreduced type Do not exceed the space provided ) <p>             This project involves longitudinal study of rhesus monkey biobehavioral ontogeny, emphasizing investigation of individual differences in behavioral and physiological reactions to mild environmental challenges and determination of long-term developmental consequences under standardized rearing conditions. Data collected this past year revealed complex but predictable relationships between genetic factors and particular postnatal experiences contributing to individual differences between same-aged monkey subjects. 1) Although nursery-reared and mother-reared juveniles achieved the same mean levels of performance on cognitive tests, the neonatal temperament characteristics of the top performers differed dramatically across the 2 rearing conditions. 2) Infants selected on the basis of genetic pedigree to be high vs. moderately reactive to challenge, then cross-fostered to multiparous females who differed in both their own reactivity and their characteristic maternal style, displayed species-normative development, with differences between infants best predicted by foster mother maternal style. However, when challenged in the absence of foster mothers differences between infants were best predicted by genetic pedigree, but when challenged in the presence of foster mothers, such differences were overridden by differences in foster mother reactivity. 3) Peer-reared infants selected on the basis of genetic pedigree showed predictable differences in behavior, HPA activity, and heart rate when introduced to an unfamiliar age-mate in a playroom setting. 4) Major differences in imipramine metabolism were found when the antidepressant was administered to adolescent monkeys under baseline vs. social challenge conditions. 5) Suppression of immune response to mitogen challenge was found in low- and mid-ranking, but not high-ranking juvenile monkeys introduced to new social groups. 6) Measures of immunoglobulins in nursery-reared, mother-reared, and foster mother-reared monkey infants revealed major developmental changes, but no rearing condition differences. Finally, 7) a pilot study of human mothers and infants obtained measures of heart rate and adrenocortical response, in addition to standardized behavioral ratings, under both control and mildly challenging circumstances.           </p>																						

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01107-03 LCE

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Adaptation of Laboratory Reared Monkeys to Field Environment

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. J. Suomi Head LCE, NICHD

Other: P. O'Neill Research Psychologist LCE, NICHD  
 G. DiGregorio Research Psychologist LCE, NICHD  
 C. Price Biologist LCE, NICHD  
 C. McKenna Psychology Aid LCE, NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Comparative Ethology

## SECTION

Comparative Behavioral Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

## TOTAL MAN-YEARS

2.20

## PROFESSIONAL

0.3

## OTHER

1.90

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (X) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves the longitudinal study of a group of 13-year-old rhesus monkeys and two generations of their progeny, all of whom live year-round in a 5-acre enclosure on the grounds of the NIHAC. The 13-year-old adults were all laboratory born, hand-reared in a nursery, and subsequently put together as a mixed-sex peer group. Despite the fact that none of these now middle-aged monkeys (nor any of their progeny) have had any physical exposure to any other monkeys, since they were first moved outdoors as juveniles they have consistently exhibited the full complement of species-normative social behavior and group organization reported to date for rhesus monkeys born and living in feral environments. During the past year major modifications were made to the monkey shelter within the 5-acre enclosure, restricting observational data collection for a 5-month period. Nevertheless, those data that were collected continued to verify the species-normative behavioral patterns in the adults and ontogenic changes in the infants, the characteristic social organization of the troop along matriarchal lines, the species-appropriate changes in social rank among troop members, most notably the drop in dominance and eventual peripheralization of adolescent males, and the successful maternal care displayed by second-generation females. In addition, another infant born outside the group was successfully cross-fostered to one of the founder females who had just delivered a still-born infant, and a study investigating the manipulative skills of transmission of tool-use-like behavior as a function of age and matriarchal lineage was initiated. Plans for a second and third enclosure progressed, with data collection initiated on some of the monkeys who will be released into the new enclosures. Finally, collaborative arrangements with the Caribbean Primate Center (Sabana Seca, Puerto Rico) were initiated for the purpose of collecting parallel data on a feral rhesus monkey troop currently living in a similar multi-acre enclosure, in order to make direct comparisons with the present study group.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01108-02 LCE
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Comparative Studies of Play Behavior		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. Bibben Senior Staff Fellow LCE, NICHD  Other: D. Symmes Head LCE, NICHD D. Bernhards Bio. Lab. Tech. LCE, NICHD G. Hetzel Animal Caretaker LCE, NICHD		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Comparative Ethology		
SECTION Section on Brain, Behavior, and Communication		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 0.8	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Two new projects on primate play, utilizing the squirrel monkey as a model, were begun this year. Data collection on both was completed in FY86.  A. <u>Social Environment Effects on Play.</u> This study investigates possible differences in the frequency and/or style of play when young squirrel monkeys are experimentally restricted to particular social companions from whom they differ with respect to age, size, or sex. Our basic strategy is to allow subject youngsters the opportunity to play only with particular partners with known play profiles. This new approach promises to yield differences in play experience without producing the devastating effects of depriving animals of social contact with peers, a flaw in previous studies performed elsewhere that has made their results ambiguous at best. The primary objective of this study is to learn the effect of different play experiences on later social outcomes. The importance of reciprocity (role reversal) in sustaining mutually beneficial play relationships may be reflected in the later development of normal and disordered cognition and behavior.  B. <u>Vocalizations Used in Play.</u> Squirrel monkeys are one of only a few species (including man) having a prominent and specific play vocalization, providing a good window for investigation of motivational changes occurring during play. Two main call types, one with four variants, were identified by sound spectrographic analysis. Vocalization rate varied with the type of ongoing behavior and with play bout duration, an association considered to be indicative of motivation to play. Structural differences also varied with bout duration, in that longer play bouts were associated with longer and more complex calls. In addition to providing direct information about motivation, vocalizations may also carry a metacommunicative message to nearby adults that the play activity is relatively harmless.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01110-01 LCE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intuitive Parenting of Infants in Comparative Perspectives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: S. J. Suomi Chief LCE, NICHD

Other: H. Papousek Visting Senior Scientist LCE, NICHD  
M. Papousek Guest Researcher LCE, NICHD  
C. Rahn Research Psychologist LCE, NICHD  
W. Thompson Guest Researcher LCE, NICHD

COOPERATING UNITS (if any)

Dept. of Psychology, University of Massachusetts (Tronick); Gallaudet Institute (Erting); Division of Maternal and Child Health, HHS (Greenspan); Laboratory of Developmental Psychobiology, Max Planck Institute, Munich

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

1.8

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present project serves two main purposes: (1) to improve knowledge on mental and communicative development in preverbal human infants, focusing attention upon didactic tendencies recently detected in intuitive forms of parental behaviors by Papousek and Papousek; and (2) to characterize similarities and differences related to intuitive parenting in two cultures--Caucasian American and Mandarin Chinese--that differ dramatically in the tonal quality of the adult language forms. Interactions between mothers and their infants at the ages of 2 and 4 months have been videotaped for microanalysis of vocal sound patterns, facial expressions, gestures, and other behaviors involved in mother-infant communication. Spectrographic analysis of vocal sounds has been methodologically enriched (in cooperation with Dr. David Symmes, BBCS, LCE) by introduction of innovative programs facilitating computer-aided analysis of pitch patterns. Data on the total of 15 American and 18 Chinese motherinfant dyads have been collected and from this data set 350 one-minute-samples have been selected macroanalytically and auditively categorized in relation to the main types of interactional contexts. Maternal utterances from these samples have been transcribed, translated by a Chinese linguist, and prepared for microanalytical evaluations in further collaboration with Papousek and Papousek at the Laboratory of Developmental Psychobiology in Munich, Germany.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 01111-01 LCE																				
PERIOD COVERED October 1, 1985 to September 30, 1986																						
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Factors Affecting Nurturant Behavior Toward Infants																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I.:</td> <td style="width: 35%;">F. A. Pedersen</td> <td style="width: 30%;">Head</td> <td style="width: 20%;">LCE, NICHD</td> </tr> <tr> <td>Other:</td> <td>G. Kestermann</td> <td>Visting Fellow</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>S. Theut</td> <td>Medical Staff Fellow</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>P. Berman</td> <td>Guest Researcher</td> <td>LCE, NICHD</td> </tr> <tr> <td></td> <td>H. Moss</td> <td>Guest Researcher</td> <td>LCE, NICHD</td> </tr> </table>			P.I.:	F. A. Pedersen	Head	LCE, NICHD	Other:	G. Kestermann	Visting Fellow	LCE, NICHD		S. Theut	Medical Staff Fellow	LCE, NICHD		P. Berman	Guest Researcher	LCE, NICHD		H. Moss	Guest Researcher	LCE, NICHD
P.I.:	F. A. Pedersen	Head	LCE, NICHD																			
Other:	G. Kestermann	Visting Fellow	LCE, NICHD																			
	S. Theut	Medical Staff Fellow	LCE, NICHD																			
	P. Berman	Guest Researcher	LCE, NICHD																			
	H. Moss	Guest Researcher	LCE, NICHD																			
COOPERATING UNITS (if any)  Rockefeller Foundation																						
LAB/BRANCH Laboratory of Comparative Ethology																						
SECTION Child and Family Research Section																						
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 2																						
TOTAL MAN-YEARS: 2.10	PROFESSIONAL: 2.10	OTHER: 0.0																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use standard unredacted type Do not exceed the space provided.) <p>             This project encompasses three recently initiated studies dealing with the development of parents' nurturant responses to infants. The first addresses the inter-generational transmission of nurturant roles for females and males. The general hypothesis being tested is that beginning very early in their children's lives, parents communicate differential expectations in their play behavior with male and female children regarding care for babies. Mothers are thought to foster stronger nurturant expectations than fathers, while fathers differentiate their role expectations for males and females more strongly than mothers do. The second study involves an intervention during the pregnancy period for first-time expectant mothers. The intervention, which includes having the expectant mother (a) handle a young infant on three different occasions, (b) observe her behavior with the infant on videotape, and (c) receive feedback about her behavior, is hypothesized to reduce anxiety, heighten the mother's sensitivity to different arousal states in the young infant, and facilitate her making appropriate responses to behavior emitted by the infant. The third study compares two groups of expectant parents who differ in exposure to a specific psychological stress, the emotional sequelae of a previous pregnancy loss, in order to determine whether the loss (miscarriage, stillbirth, or neonatal death) contributes toward anxiety, depression, and a dysfunctional parental adaptation that can interfere with nurturant behavior toward the young. For each study the pilot phase has been completed and data collection is in progress.           </p>																						

LABORATORY OF DEVELOPMENTAL AND MOLECULAR IMMUNITY

- Z01 HD 00073-15    Regulation of Immune Systems at the Cellular Level  
                         Edgar E. Hanna, Ph.D.
- Z01 HD 00918-05    Expression of Histocompatibility Antigens During  
                         Early Mammalian Development  
                         Keiko Ozato, Ph.D.
- Z01 HD 00920-05    Molecular Structure of Mouse Histocompatibility  
                         (H-2) Genes:  
                         Keiko Ozato, Ph.D.
- Z01 HD 01301-04    Human Immune Response to Polysaccharide-Protein  
                         Conjugate Vaccines  
                         Rachel Schneerson, M.D.
- Z01 HD 01302-04    Toxins of Pertussis: Isolation, Characterization and  
                         Mechanisms of Action  
                         Ronald D. Sekura, Ph.D.
- Z01 HD 01304-04    Protective Effect of Vi Polysaccharide Antibodies Against  
                         Typhoid Fever  
                         John B. Robbins, M.D.
- Z01 HD 01306-03    Pertussis Heat Labile Toxin (HLT): Isolation and  
                         Characterization  
                         Ronald D. Sekura, Ph.D.
- Z01 HD 01307-03    Pertussis Toxin: An Approach to a New Pertussis Vaccine  
                         Ronald D. Sekura, Ph.D.
- Z01 HD 01308-03    Conjugation of Pneumococcal Vi Polysaccharides with  
                         Carrier Proteins  
                         Shousun. C. Szu, Ph.D.
- Z01 HD 01309-03    Bacterial Polysaccharides Cross-Reactive with Meningococcal  
                         Group A Polysaccharide  
                         R. Schneerson, M.D.





NICHD ANNUAL REPORT  
October 1, 1985 to September 30, 1986

Laboratory of Developmental and Molecular Immunity

Research is conducted into developmental and molecular biology of "natural," disease-acquired and immunization-induced immunity with especial emphasis on antigens involved in the immunopathogenesis of diseases of the fetus, newborn infant and young child. Three areas of inquiry have been studied; 1) Immuno-pathogenesis of invasive diseases of infants and young children due to encapsulated bacteria development of vaccines for their prevention, and characterization of the mechanisms underlying the age-related development of serum antibody formation to the protective antigens of these pathogens. Methods are under study in order to provide more rapid and reliable diagnosis of bacterial infections of infants. The exotoxins of Bordetella pertussis, including pertussis toxin, are studied for their immunopathogenic role in pertussis; 2) The development, activation, timing of expression, regulation of intrauterine and neonatal synthesis and structure-function relations of the Class I transplantation antigens. Site-directed mutagenesis is used to provide information about the structure function of these polymorphic immunoregulatory molecules and the effect of interferon, retinoic acid and oncogenes upon the synthesis of Class I histocompatibility antigens is being studied by a variety of methods including the novel approach of using cell cultures taken from embryos at crucial periods of the development of these antigens. Critical to this program is the elucidation of the DNA sequences surrounding the Class I MHC antigens involved in their structure, regulation and intrauterine activation; 3) The immunoregulatory function of bacterial toxins, including pertussis toxin and Group A beta hemolytic streptococcal pyrogenic exotoxin, is being characterized using hybridomas constructed from both T and B lymphoid cells at various stages of their differentiation.

Schneerson and Robbins continue their work on the pathogenesis and prevention of invasive diseases due to encapsulated bacteria of infants and children. Their emphasis has been primarily on Haemophilus influenzae type b and pneumococcal infections, but has now been extended to typhoid fever and related causes of enteric fevers.

The effectiveness of the H. influenzae type b and pneumococcal polysaccharide vaccines in infants and young children is hampered by their age-related immunogenicity and lack of a booster response upon reinjection. Synthetic schemes were devised by Schneerson and Robbins in order to both increase the immunogenicity of and confer the properties of T cell dependence to the capsular polysaccharides of H. influenzae type b and pneumococcus type 6 (as an example of the weakest immunogen and age-dependent component of the pneumococcal vaccine). Following extensive studies of the immunologic and safety properties of these vaccines in laboratory rodents and then primates, a clinical study in adults was completed. Volunteers were immunized two times with conjugates composed of H. influenzae type b, the cross-reacting Escherichia coli K100 or pneumococcal type 6A polysaccharides bound to tetanus toxoid. Local reactions were common and probably due to Arthus reactions mediated by high levels of preexisting tetanus toxin antibodies.

Fever occurred in about 10% of recipients after the first injection; none was detected after the second injection. The H. influenzae type b-tetanus toxoid conjugate elicited a 180-fold increase in serum polysaccharide antibodies and the pneumococcus type 6A conjugate elicited an 8-fold increase. Each conjugate elicited about a 10- to 20-fold increase in serum tetanus toxin antibodies. Similar levels of antibodies to the polysaccharide and tetanus toxoid components were elicited by 50 and 100 microgram doses and by one conjugate or combination of conjugates. Pre- and post-immunization sera had H. influenzae type b antibodies composed of IgG, IgA and IgM immunoglobulins. Immunization with either the purified polysaccharide or the conjugates elicited increase in all three isotypes; the greatest increase was in the IgG component. The highest responses in this latter isotype was in the IgG2 subclass. A one way cross-reaction was noted as about one-half of volunteers immunized with the pneumococcus type 6A conjugate responded with 2-fold or greater levels of H. influenzae type b antibodies. The polysaccharide and tetanus toxoid antibodies elicited by the conjugates exerted biological activities that have been correlated with immunity to infection with their respective agents. The conclusion from these first clinical studies is that the quality of antibodies elicited by the conjugate is similar to that elicited by the component injected alone; the level of polysaccharide antibodies stimulated by the conjugates was considerably higher. Phase II studies of these conjugates in children are ongoing.

Preliminary evaluation of these conjugates in patients with immunodeficiencies and recurrent infections has been done in collaboration with Dr. Lars A. Hanson, University of Goteborg. Most patients responded with levels of H. influenzae type b antibodies similar to the normal volunteers; two patients with non-detectable or very low levels of IgG2 responded considerably lower, though at levels considered to be protective. Another collaborative study, with Drs. Sarnack and Kaplan of Wayne State University, is planned to evaluate these conjugates in patients of the infant and child age group with sickle cell anemia, another condition that predisposes to an unusually high rate of invasive infections with encapsulated bacteria.

Similar to other encapsulated bacterial pathogens, capsular polysaccharide antibodies confer immunity to Neisseria meningitidis. In contrast to the other serogroups, meningococcal Group A diseases have a unique epidemiology; in Africa, Group A meningitis is endemic with high frequency. In other parts of the world, Group A causes epidemics. In both situations, asymptomatic carriage is low. In the U.S., Group A meningococci have not been detected for the past 30 years. Most young adults have Group A polysaccharide antibodies. Yet, the antigenic stimuli for these antibodies has not been identified. Two Escherichia coli capsular polysaccharides, K93 and K51, were discovered which could account for this ubiquitous "natural" immunity. Their serological properties and structures were characterized. Schemes for synthesis of K93, K51 and Group A polysaccharide conjugates have been devised in order to provide more effective immunogens against Group A meningitis in infants and children than provided by the current Group A vaccine.

A patient, without malignancy and with high levels of a monoclonal antibody reactive with both Group B meningococcal and E. coli K1 capsular

polysaccharides, was discovered. The specificity and protective effects of this monoclonal antibody were characterized. Its therapeutic effect in the treatment of Group B meningococcal meningitis is planned to be studied.

Pneumococcal infections cause considerable morbidity and continuing mortality, especially in infants and young children, despite effective antibiotics and supportive care. The limitations of the pneumococcal vaccine (vide supra) prompted a search for a more immunogenic and species-specific, rather than a multivalent type-specific, immunogen. Briles, et al., showed that monoclonal antibodies, specific for phosphocholine (PC) conferred protection in mice against several pneumococcal types. The most likely candidate for this PC-containing protective antigen was the cell wall polysaccharide (Cw-Ps). The Cw-Ps is present in all pneumococci, is a linear copolymer which contains PC bound to its galactose residue. The Cw-Ps was bound to a carrier protein through the use of a heterobifunctional coupling agent, N-succinimidyl 3-(2-pyridyldithio-propionate (SPDP). The resultant Cw-Ps protein conjugate was injected into animals and the hyperimmune antisera assayed for its ability to confer passive protection against lethal challenge with several pneumococcal types in a standardized mouse model. The conjugate elicited Cw-Ps antibodies without PC specificity and did not confer protection. Cw-Ps antibodies elicited by multiple intravenous injection of a mutant pneumococcus strain, SRC-2, which contains a capsule composed of the Cw-Ps, elicited antibodies with PC specificity. This antiserum was protective, but, absorption with Cw-Ps did not change its protective activity. These data provide evidence that antibodies to the Cw-Ps do not confer immunity against infection of mice with encapsulated pneumococci. A search for the protective activities of other PC-containing structures is ongoing.

Enteric fevers have a similar pathogenesis, clinical picture and are caused by Salmonellae. In developing nations, the most common, usually accounting for about 80%, and most severe is typhoid fever. Typhoid fever continues to be a problem for U.S. citizens in countries where typhoid fever is epidemic. Transmission of Salmonella typhi, the causative agent of typhoid fever, is almost always by contaminated water. Control of enteric fevers has been achieved where practices of hygiene have been applied to food handling, disposal of sewage, identification of patients, etc. It is unlikely that control will be achieved in developing nations in the near future. One method of control is vaccination. Our current typhoid vaccines are unsatisfactory because they induce a high rate of side reactions and confer only a limited immunity.

S. typhi is pathogenic for humans only; there are no satisfactory animal models. S. typhi has a capsular polysaccharide, called the VI (virulence) antigen; no other Salmonellae has a comparable structure. Circumstantial evidence has been amassed to suggest that antibodies to the VI antigen confer immunity to typhoid fever. Accordingly, methods were devised for the production, isolation, standardization, and evaluation of the effectiveness of the VI polysaccharide to prevent typhoid fever.

The VI vaccine was standardized by methods established for other polysaccharides and by <sup>14</sup>C nuclear magnetic resonance spectroscopy.



Clinical safety and immunogenicity conducted in medical students in Lyon, France, and in school children and adults, ages 5 through 45 years in various villages of the Kathmandu Valley, Nepal, were considered satisfactory.

Accordingly, effectiveness of the Vi polysaccharide to prevent typhoid fever was evaluated in a clinical study established in five villages, comprising a total population of about 13,000, in Nepal. Seven thousand volunteered and received either 25 micrograms of the Vi polysaccharide or the 23 valent pneumococcal vaccine (control) in a randomized and double-masked fashion. No serious reactions were encountered. Local swelling and redness occurred in about 10% of the vaccinates, more noticeably in the adults. The reactions were not incapacitating and waned in 48 hours. Their timing and appearance were consistent with the reactivity elicited by pneumococcal vaccines in adults.

Surveillance will be maintained in the five villages that participated in the effectiveness program for at least two years. If effectiveness is demonstrated, we plan to request funds from the Agency for International Development, to maintain this surveillance for an additional five years to ascertain the duration of immunity. As the routine of surveillance becomes established, we hope to study the genetic regulation and other immunological characteristics of the patients with typhoid fever.

It is stated that typhoid fever is unusual under the age of five years. This is unexpected because other infectious diseases, contracted by ingestion of contaminated water, occur with high frequency in this age group. We plan to study the epidemiology of typhoid fever and other causes of enteric fevers in this age group using more reliable methods of diagnosis including bone marrow culture.

Based upon these studies and the results of our effectiveness study in Nepal, we will judge whether or not the Vi alone could be studied for its effectiveness to prevent typhoid fever in infants and children up to the age of five years. We have developed methods for binding capsular polysaccharides that contain an uronic acid residue (e.g., the Vi polysaccharide) to medically useful carrier proteins in order to make them effective vaccines for infants and young children. The safety, immunogenicity, and then effectiveness of our newly synthesized Vi-protein conjugates could then be considered for evaluation in the very young.

The availability of safe and effective typhoid vaccine will not eliminate enteric fevers due to the other species of Salmonellae. The antigens of the other Salmonella reside in their lipopolysaccharide (LPS). LPS antigens must be modified into both non-toxic and immunogenic forms. A vaccine, composed of protein conjugates of the Vi capsular polysaccharide and one or several detoxified LPS, could be both compatible in a single formulation alone or with DTP.

The incidence of pertussis, a severe and communicable disease with its high morbidity and mortality in infants and young children, has been controlled by vaccination. Our current pertussis vaccine is composed of inactivated Bordetella pertussis organisms and is a component of the triple formulation,

DTP, given routinely to infants and children. The pertussis component, however, suffers from two limitations; 1) It induces a high rate of side reactions, including a rare encephalopathy; 2) The extent and duration of protection are limited compared to other vaccines. The side reactions have caused public concern and the use and supply of pertussis vaccines is decreasing. Recently, Pittman proposed that a single component of B. pertussis, pertussis toxin (PT), was a major immunopathogenic determinant. We concur with Pittman's proposal that pertussis is a toxin-mediated disease and that serum PT antibodies will confer immunity. Accordingly, methods for production of PT in quantities and purity suitable for clinical investigation were developed by Sekura. PT has an enzymatic action, ADP ribosylation, similar to other bacterial exotoxins including that of C. diphtheriae and V. cholera. The specificity of binding of PT to mammalian cells was studied using the serum glycoprotein, fetuin, as a model compound. Binding of PT to fetuin was shown to be due to its interaction with the asparagine-linked oligosaccharide of this glycoprotein. A novel method was devised in order to inactivate both the binding and enzymatic activities of PT while retaining at least one-third of its antigenicity. The detoxified PT induced active immunity against challenge with B. pertussis by the intracerebral and pulmonary routes in laboratory mice. A lot of Ptd, PTH-06, prepared and standardized for clinical evaluation, showed no toxicity and induced neutralizing antibodies in primates. Assays to monitor the antigenicity, immunogenicity, binding and enzymatic activity of the inactivated PT (pertussis toxoid or PTd), that could be used for routine standardization by control agencies, were developed and their usefulness and reliability confirmed with several production lots. Clinical evaluation was started in adult volunteers at the Clinical Center of the NIH. Three doses, ranging from 10 to 75 ug of PTd adsorbed on aluminum salts, were injected into 25 volunteers each. No local or systemic reactions, alteration in glucose metabolism or changes in lymphocyte counts were observed. Serological analyses are ongoing. It is planned to start Phase II studies in children in Goteborg, Sweden, in the next several months assuming the the serum antibody responses elicited by the experimental PTd are similar to those observed in mice and primates.

B. pertussis is a pathogen for humans only; clinical pertussis has not been established in other animals. In addition to PT, this pathogen secretes a variety of biologically active extracellular materials, including adenylate cyclase and an activity referred to as dermonecrotic or heat labile toxin (HLT). This later activity has not been characterized. Sekura and his associates have isolated a protein toxin from sonicates of B. pertussis which have this activity. Homogenous preparations of HLT have been obtained and it was established that the toxin is a polypeptide with a molecular weight of about 150,000.

Purified HLT has been used to produce polyclonal antibodies in rabbits and monoclonal antibodies from hybridoma cultures. Passive immunization of mice with polyclonal HLT antibodies conferred protection against challenge with B. pertussis suggesting that HLT may serve as an additional protective antigen. Sublethal doses of HLT, injected into mice, produced unexpected results. Within eight hours, about a 70% reduction in the weight of the spleen was observed. Most of the loss in spleen weight was due to reduction in the red

pulp (erythropoietic tissue). The lethal dose of HLT in mice ranged about 0.5 ug, similar to the toxicity of tetanus toxin. HLT antibodies conferred protection against the lethal effects of toxin challenge (anti-toxin).

Sekura speculates that HLT induces local inflammation at the site of initial infection with B. pertussis. This local inflammatory action may account for the early non-specific respiratory symptoms of infection with B. pertussis. Studies are underway to both measure HLT antibodies in patients with pertussis and to assess its potential for inducing protective immunity.

Ozato continues to unearth new information about the structure-function relations and developmental regulation of the Major Histocompatibility Class I antigens (MHC). These cell surface molecules are polymorphic membrane glycoproteins involved in tissue rejection and cell-bound antigen recognition. Class I MHC antigens are composed of three external domains and one membrane-associated domain. The notable structural features of MHC Class I genes are their extraordinary degree of polymorphism. This high level of polymorphism has been maintained throughout evolution of the vertebrates and cannot be explained by simple genetic drift. MHC Class I antigens present cell-bound antigens in order to stimulate T cells. This activity is mediated by the polymorphic portion since Class I MHC mismatched cells fail to stimulate T cells. Variability is found in Class I MHC genes throughout the first and second domains making it difficult to identify the regions responsible for its immunological functions.

Site directed mutagenesis was used to study the structure-function relations of Class I MHC genes. Mutant H.2.Ld genes were prepared in which a few amino acids in the most polymorphic portions of the first external domain were replaced by amino acids of other Class I MHC genes. A 1.9 kb Xba fragment of the H-2L<sup>d</sup> gene, containing the first and second exons cloned in M13, served as a template. Synthetic nucleotides of 27 base pairs, containing 2 to 3 nucleotide mismatches, were annealed to the templates and the mutants were identified by dot hybridization. The putative mutants were analyzed by DNA sequencing. The mutants, once identified, were introduced into murine L cells by co-infection with Herpes thymidine kinase gene. Radioimmuno-binding, cytofluorography and reactivity with CTL lymphocytes were used to analyze the expression of these mutant genes. This approach has been very productive; Ozato, et al., found a small stretch of amino acid sequences is responsible for a number of antigenic determinants recognized by monoclonal antibodies and by CTL. Also, changes in glycosylation sites and disulphide bridges induced by this system have already produced information about the location of antigenic sites and intracellular transportation of these antigens.

Ozato found that murine alpha, beta and gamma interferons (IFNs) induce c-fos oncogene expression in undifferentiated embryonal teratocarcinoma F9 EC cells, NIH 3T3 cells, and a variety of lymphoid cells. IFNs-induced expression is dose-dependent, rapid and transient; the amount of c-fos transcripts rises within 15 minutes, peaks after 30 minutes, and then soon drops to an undetectable level. The c-fos gene reveals a stretch of 10 nucleotides in the 5' flanking region of the gene homologous to a part of an "IFN-consensus sequence." This, and other findings, indicate that c-fos is a



member of IFN-inducible genes and that its expression is an early event of IFN action. C-fos mRNA increases steeply in several tissues on the day of birth followed by a precipitous decline soon thereafter. Immediately following this burst of c-fos expression, the level of MHC Class I RNA, known to be induced by IFNs, rises sharply in these neonatal organs. These findings suggest that the sequential expression of c-fos and MHC Class I genes are regulated by IFNs which are an integral component of neonatal development.

Murine MHC Class I genes are expressed only after embryos reach mid-somite stage. There are no established cell lines representative of embryos at their mid-somite stage. Accordingly, a number of clones from mouse embryos at day 8 and day 11 were established by infection and transformation with several oncogenic retroviruses. These clones of embryonic cells are morphologically distinct. Of 35 independent clones, 10 have been characterized further and found to have different expression of MHC Class I and SSEA-1 antigens and laminine. SSEA-1 is expressed in early embryonic cells and is no longer detectable after differentiation. Laminine has been shown to be undetectable in early embryos; it is expressed in epithelial cells and later embryos. All ten clones were negative for SSEA-1. Laminine was detected in only some of these 10 clones. Unlike F9 EC cells, these clones did not show morphological changes after treatment with retinoic acid. These cells, therefore, appear to retain the characteristics of mid-gestational embryos. The majority of these clones have low levels of MHC Class I mRNA equivalent to F9 EC cells. Several clones negative for surface MHC Class I antigens showed relatively high constitutive expression MHC of Class I mRNA. Interferon treatment of these clones resulted in increase in the level of MHC Class I antigens. The kinetics of their IFN-induced MHC Class I surface antigens and mRNA were variable. CAT assay of these clones for a negative regulatory factor, found in the undifferentiated F9 EC cells are underway.

The development and expression of MHC Class I antigens was analyzed with F9 EC cells to study their gene regulation. The promoter activity of MHC Class I genes was analyzed by employing chloramphenicol acetyl transferase (CAT) fusion genes in a transient transfection system.

Sequence data was obtained from the upstream region of the H-2L<sup>d</sup> gene in order to discover controlling elements. Portions of its upstream region were fused to the coding sequence of the bacterial CAT gene. Another series of CAT constructs was prepared in which portions of the SV40 promoter have been replaced by the MHC Class I promoter sequences. The promoter activity of the above constructs was assessed in F9 EC cells by measuring transient CAT activity after DNA-mediated gene transfer.

The promoter activity of a MHC Class I gene was studied with F9 EC cells, employing CAT assays with hybrid genes containing upstream regions of a MHC Class I H-2L<sup>d</sup> gene. The 1.4 kb 5' flanking region of the H-2L<sup>d</sup> gene revealed the presence of 5-10 bp long direct or inverted repeats about 100-230 bp upstream of the gene immediately adjacent to the IFN consensus sequence. Chimeric genes, in which the CAT genes were under the control of various lengths of 5' flanking region of the H-2L<sup>d</sup> gene, were introduced in F9 EC



cells and the promoter activity was estimated by measuring transient expression of the CAT gene. We found the upstream region of the H-2L<sup>d</sup> gene has weak promoter activity in F9 EC cells. When the upstream sequence, distal from position 135 (from the cap site) was removed, a 4- to 5-fold increase in CAT activity ensued. Studies with internal deletions indicated that the presence of a 75 bp long sequence encompassing nucleotide position -135 to -210 bp from the cap site, represses CAT activity in undifferentiated F9 cells. Upstream fragments that lack this region consistently elicited 4- to 5-fold higher CAT activity. This repression was demonstrable in undifferentiated F9 cells only; all the constructs had similar CAT activity when the F9 cells had been differentiated by treatment with retinoic acid. This finding indicates that the repressed promoter of the MHC Class I gene is developmentally regulated. The region, responsible for this repression, contained inverted and direct repeats consisting of 5 - 10 bp nucleotides and the IFN consensus sequence in the proximal region, exerts enhancer-like activity in differentiated cells only.

Ozato will continue to study developmental regulation of MHC Class I genes. Her working hypothesis is that the regulatory region in the 5' flanking region of the H-2L<sup>d</sup> gene interacts with a transacting nuclear protein(s) present in early embryonic cells. Proto-oncogene, c-fos, is expressed during murine embryonic development. The c-fos gene is also induced by other growth stimuli in many tissue culture cells. Although c-fos has been implicated to be involved in differentiation, no precise function of this regulatory effect has been demonstrated. Ozato proposes to introduce c-fos gene, under the control of the H-2L<sup>d</sup> promoter, into fertilized mouse embryos and produce transgenic mice. Construction of this hybrid gene has been completed and its expression is being evaluated in F9 EC cells to confirm its predicted development. This construct will be introduced into C57BL/6 embryos which are then allowed to develop in foster mothers. The information derived from this new model will be evaluated with the DNA analysis of cells in culture including the embryonal cell cultures developed by this laboratory.

During the course of systemic infections, lymphoid function towards the offending agent and other antigens is often altered (deregulated). In many instances, this deregulation may affect the primary course of the disease or predispose to secondary complications. Hanna and his associates study how microorganisms or their products alter the regulation of lymphoid tissue structure and function. Hybridomas have been constructed from spleen cell cultures which represent precursor and mature regulatory T-cells. The spleen cell cultures, used to supply the functional cell type of the hybridoma, were either stimulated by T-cell growth factors, antigens and fractionated into homogenous cell types prior to the fusion. Cell surface architecture is analyzed by dual laser, computer-controlled cell sorter/analyzer with fluorochrome-labeled monoclonal antibodies. Lymphoid function is measured by a variety of in vitro and in vivo standardized techniques including generation and function of cytotoxic T lymphocytes (CTL) and antibody formation. Two bacterial products have been studied in detail; pertussis toxin (PT) and streptococcal pyrogenic exotoxin (SPE). Both toxins exert both stimulatory and suppressive activities on various immune functions when injected in vivo; these effects are complex and depend upon the dose, timing

and type of immune function that are assayed. Both protein toxins are mitogenic at low concentrations and not cytotoxic at doses that induce effects upon lymphoid function. Both toxins are thought to exert a major immunopathogenic role during diseases caused by their parent bacteria. SPE exerts its effect upon precursor down regulatory T-cells for antibody formation. Exposure of one hybridoma, a precursor T-cell with the differentiation markers Thyl, L3T4 and Lyt2, results in reduction of its suppressive activity. The SPE-induced change of this precursor T-cell hybridoma results in reduced expression of both its Lyt2 marker and suppressor activity for antibody formation by B-lymphocytes. This effect is consistent with the capacity of SPE to divert development of function in T-cell precursors. The SPE-induced suppression of CTL, in contrast, was not as marked as that mediated by PT. PT exerts its immunomodulating effect by generating an increase in suppressor cell types. Spleen cell cultures, exposed to this toxin, show an increase in LsT4<sup>(-)</sup>, Lyt2<sup>+</sup>, Thyl<sup>+</sup>, T-cells. These cells have been partially purified and found to exert a suppressive effect upon generation of CTL's in cell culture.

Stress mimicking compounds, such as N'methyl-B-carboline-3-carboxamide, exert modulatory effects upon lymphoid function. Treatment of animals with this compound resulted in suppressed generation of CTL. This in vivo effect was not demonstrated by direct addition to spleen cultures for generating CTL. The intermediate biological mediator of this suppressive effect remains to be elucidated. Hanna and his colleagues are seeking an understanding of the biological intermediates and will employ his library of precursor and T-cell clones and hybridomas to identify the cellular basis for this immunomodulating effect.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00073-15 LDMI

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Immune Systems at the Cellular Level

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Edgar Hanna Head LDMI, NICHD

Others: Prince Arora Staff Fellow LDMI, NICHD  
Jin-Su Choi Visiting Fellow LDMI, NICHD  
Michael Walker Biologist (Tech) LDMI, NICHD  
Keiko Ozato Research Microbiologist LDMI, NICHD  
Ronald Sekura Research Chemist LDMI, NICHD

## COOPERATING UNITS (if any)

P. Skolnick, LBC, NIDDK; C. Hansen, R VR

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Immunoregulation and Cellular Control

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

3.0

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to understand how microorganisms or their products modulate or deregulate immune systems, we have constructed monoclonal lines of immunocytes as cloned hybridomas. These express the phenotypes of various fractionated splenocytes. Hybridomas, representing mature and precursor regulatory T-cells, have been constructed from NFR/N mice and from NFR-nude mice respectively. Cytotoxic T-cells (CTL) have been cloned from 5-day splenocyte cultures in the presence of T-cell growth factors and antigen. We have used the toxins, streptococcal pyrogenic exotoxin (SPE) and pertussis toxin (PT) in parallel to study their immunomodulatory activities *in vitro*. We have shown that SPE interferes with the normal down regulation of T-cell dependent antibody responses. In experiments employing mature-type and precursor-type monoclonal T-cell clones, we observe that the cell generating the SPE effect is a precursor, rather than a mature functional T-cell. Whereas, the mature-type suppressor clones are antigen (carrier)-specific, the precursor-type clones are non-specific, non-NK, suppressor clones. One of these clones expressed the T-cell differentiation markers L3T4 and Lyt2 simultaneously in flow cytometry. A subclone has been derived from growth in the presence of SPE with reduced suppressive activity and reduced expression of Lyt2. The change appears to be a sustained, phenotypic change through several generations. We have been unable, so far, to ascertain rearrangements in the alpha or beta chain gene of the T-cell receptor in either of these nude mouse T-cell hybridomas. SPE and PT are inhibitory to CTL responses; PT is about 10-fold more inhibitory than SPE. SPE had been shown to be only transiently inhibitory in PFC assays. Both toxins are active without cytotoxic effects. They are mitogenic at low concentrations. PT generates a cell-associated CTL suppressive activity in splenocyte cultures and an increased number of L3T4<sup>(-)</sup>, Lyt2<sup>+</sup>, Thy1<sup>+</sup> T-cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00918-05 LDMI																																
PERIOD COVERED October 1, 1985 to September 30, 1986																																		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Expression of Histocompatibility Antigens During Early Mammalian Development																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Keiko Ozato</td> <td style="width: 25%;">Head</td> <td style="width: 20%;">LDMI, NICHD</td> </tr> <tr> <td colspan="4"> </td> </tr> <tr> <td>Others:</td> <td>Jun-ichi Miyazaki</td> <td>Visiting Associate</td> <td>LDMI, NICHD</td> </tr> <tr> <td></td> <td>John Kasik</td> <td>Guest Researcher</td> <td>LDMI, NICHD</td> </tr> <tr> <td></td> <td>Kenji Sugita</td> <td>Guest Researcher</td> <td>LDMI, NICHD</td> </tr> <tr> <td></td> <td>Ben-Zion Levi</td> <td>Visiting Fellow</td> <td>LDMI, NICHD</td> </tr> <tr> <td></td> <td>Toby Silverman</td> <td>Guest Researcher</td> <td>LDMI, NICHD</td> </tr> <tr> <td></td> <td>Bonnie Orrison</td> <td>Chemist</td> <td>LDMI, NICHD</td> </tr> </table>			PI:	Keiko Ozato	Head	LDMI, NICHD					Others:	Jun-ichi Miyazaki	Visiting Associate	LDMI, NICHD		John Kasik	Guest Researcher	LDMI, NICHD		Kenji Sugita	Guest Researcher	LDMI, NICHD		Ben-Zion Levi	Visiting Fellow	LDMI, NICHD		Toby Silverman	Guest Researcher	LDMI, NICHD		Bonnie Orrison	Chemist	LDMI, NICHD
PI:	Keiko Ozato	Head	LDMI, NICHD																															
Others:	Jun-ichi Miyazaki	Visiting Associate	LDMI, NICHD																															
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	Toby Silverman	Guest Researcher	LDMI, NICHD																															
	Bonnie Orrison	Chemist	LDMI, NICHD																															
COOPERATING UNITS (if any)  None																																		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity																																		
SECTION Unit on Molecular Genetics of Immunity																																		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892																																		
TOTAL MAN-YEARS: 6.5	PROFESSIONAL 5.5	OTHER: 1.0																																
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)  <p>           Developmental regulation of MHC Class I gene expression has been studied <u>in vitro</u> with F9 embryonal teratocarcinoma (EC) cells. Undifferentiated F9 EC cells do not express the MHC antigens, while these cells treated with retinoic acid, or interferons, accumulate both MHC Class I mRNA and the MHC Class I antigens. We have constructed chloramphenicol acetyl transferase (CAT) genes connected to various portions of the 5' flanking region of a mouse MHC Class I gene in F9 EC cells. We found that the upstream sequence of the MHC Class I antigen contains a sequence which represses the promoter activity of the gene in undifferentiated F9 cells. The repression was no longer found when F9 EC cells are differentiated with retinoic acid. Studies of constructs containing internal deletions allowed us to map the sequence controlling the repression to -130 to -210 bp upstream from the cap site. When a fragment containing this "regulatory sequence" was placed in the upstream of a SV40 promoter, the characteristic repression of the CAT activity was observed in undifferentiated F9 EC cells. This repression was relieved upon differentiation of the EC cells. We postulate that developmental regulation of MHC Class I genes is controlled, in part, by this regulatory sequence. In a separate study, we found that interferons (IFNs) induce proto-oncogene <u>c-fos</u> in a variety of tissue culture cells. The induction of <u>c-fos</u> mRNA is rapid and transient occurring within 15 minutes of IFN treatment; the elevated levels return to basal levels within 2 hours. <u>c-fos</u> and Class I mRNA are thus induced in a sequential manner by IFN treatment in tissue cultures. Moreover, <u>c-fos</u> is found to be transiently expressed in several tissues during murine neonatal development. Sharp increases in the level of MHC Class I mRNA occur immediately following this event, suggesting that IFNs may play a role in post-natal mammalian development.         </p>																																		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00920-05 LDMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Structure of Mouse Histocompatibility (H-2) Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div>PI: Keiko Ozato</div> <div>Head</div> <div>LDMI, NICHD</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Others: David Koeller</div> <div>Medical Staff Fellow</div> <div>LDMI, NICHD</div> </div>		
COOPERATING UNITS (if any) E. Appella, LCB, NCI; J. Frelinger, University of North Carolina Medical School, Chapel Hill, North Carolina		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity		
SECTION Unit on Molecular Genetics of Immunity		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           MHC Class I antigens are polymorphic membrane glycoproteins involved in tissue rejection. This project aims to analyze structure-function relationships of these antigens. Employing site-directed mutagenesis, we have studied the polymorphic sites involved in the immune functions of these antigens. Four step mutagenesis has been undertaken to introduce five amino acid substitutions in position 63-73 of the mouse H-2L<sup>d</sup> antigen. These positions are replaced by the H-2D<sup>d</sup> type amino acids in the mutant antigens. Our rationale for this mutagenesis is based on our initial efforts to predict immunologically functional sites of the MHC Class I antigens. The mutagenesis to replace four amino acids is complete, and the mutated genes have been introduced into L-cells. We plan to characterize functions of the mutated H-2L<sup>d</sup> gene products by testing their reactivities with more than 50 monoclonal antibodies. Further, we plan to test reactivities of T-cells specific for using H-2 restricted cytotoxic T-cells (CTL), and alloreactive CTLs. In a collaborative study, we have sequenced the H-2DP gene located from B10 mice. The H-2P haplotype is remote in its origin from other laboratory strains. We found the H-2DP gene is very homologous to known MHC Class I genes, the highest homology being found with the H-2L<sup>d</sup> and H-2D<sup>b</sup> genes, rather than other D region genes. Unlike most other structural genes, greater numbers of nucleotide substitutions are found in exons than in introns suggesting evolutionary force to increase the polymorphism. Chimeric Class I genes were constructed which have the first and the second domain sequences derived from two different genes, DP or D<sup>d</sup>. Characterization of transformants is underway to study contributions of conformational structures generated by both domains to antigenicity and to functions of Class I antigens.         </p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01301-04 LDMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Immune Response to Polysaccharide-Protein Conjugate Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Rachel Schneerson	Senior Investigator LDMI, NICHD
Others:	John B. Robbins Yong Hong Yang	Head LDMI, NICHD Visiting Fellow LDMI, NICHD
COOPERATING UNITS (if any) G. Schiffman, State University, New York; J.C. Parke, Jr., Charlotte Memorial Hospital, North Carolina; J. Schlesselman, USUHS, Bethesda, Maryland; C. Bell, University of Illinois at Chicago		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity		
SECTION Section on Bacterial Disease Pathogenesis and Immunity		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.9	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Haemophilus influenzae</u> type b (Hib) is the leading cause of bacterial meningitis in infants and children and a major cause of septicemia, septic arthritis pneumonia and epiglottitis. <u>Pneumococcus</u> type 6A (Pn6A) is a major cause of otitis media and the most frequent type causing meningitis pneumonia. Anticapsular antibodies to these two pathogens are protective; their induction in the young by capsular polysaccharides is hampered by both their age-related immunogenicity and lack of anamnetic response. In contrast, conjugates composed of these polysaccharides covalently bound to tetanus toxoid were immunogenic in mice and infant rhesus; this response could be boosted by further injections. Adult volunteers were immunized two times at three week intervals with conjugates composed of Hib, <u>Escherichia coli</u> K100, or Pn6A polysaccharides and tetanus toxoid. Local reactions were common and probably due to Arthus reaction by preexisting tetanus antitoxin. Fever occurred in ten of the recipients after the first injection; in none after the second injection. Hib-TT elicited a 180-fold increase in Hib antibody. Pn6A-TT elicited about an 8-fold increase in Pn6A antibody. Each conjugate induced a 10 to 20-fold increase in TT antibodies. A maximal response occurred in most volunteers after the first injection, with no booster response observed after the second injection. No relation was found between the pre-immune levels with the rate of antibody rise or to the side effects of the vaccines. Similar antibody levels were induced by 50 or 100 ug doses and by one conjugate or combination of conjugates. Pre and post-immunization sera had Hib antibodies composed of IgG, IgM, and IgA. Immunization produced the highest response in IgG. Similarly, pre and post-immunization sera were distributed among all IgG subclasses, with the highest response in the IgG2. Pn6A-TT and Hib-TT have been prepared and immunization of 18 month to 2 year old children has begun in Goteborg, Sweden.         </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01302-04 LDMI

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Toxins of Pertussis: Isolation, Characterization, and Mechanisms of Action

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald Sekura	Research Chemist	LDMI, NICHD
Others:	Yan-ling Zhang	Visiting Associate	LDMI, NICHD
	Nathaniel Tolson	Biologist	LDMI, NICHD
	Edgar Hanna	Research Microbiologist	LDMI, NICHD
	Prince Arora	Staff Fellow	LDMI, NICHD

## COOPERATING UNITS (if any)

T. Cote, USUHS, Bethesda, Maryland; E. Hanna, NICHD

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Bacterial Disease and Pathogenesis and Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.8

## PROFESSIONAL:

0.3

## OTHER

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Bordetella pertussis, the microorganisms which cause the disease commonly known as whooping cough, produces several toxins (i.e., pertussis toxin (PT) and heat labile or dermonecrotic toxin) (see project Z01 HD 01306) which appear to play important roles in pathogenesis of the organism. PT, in addition, is a major protective antigen which is a promising candidate for the development of a new acellular pertussis vaccine (see project Z01 HD 01307). The current project concentrates on elucidating the mechanisms by which pertussis toxin interacts with cells and elicits its diverse pharmacologic actions. The initial event in the interaction of PT with cells appears to be a rapid and essentially irreversible binding of toxin to cells. Using the interaction of PT with fetuin as a model, studies have been conducted which identify the carbohydrate structure with which pertussis toxin interacts. The toxic action of PT is mediated by toxin catalyzed transfer of the ADP-ribose moiety from NAD to the adenylate cyclase regulatory component,  $N_i$ . This action of PT has been used as a probe to explore the role of  $N_i$  in regulation of cell function, with specific emphasis on those cell types involved in immune response. Treatment of lymphoid cells with PT results in diverse responses which establish that in pathogenesis, PT acts to interfere with the immune response. Treatment with PT has been shown to block the production of cytotoxic T-lymphocytes (CTL's) and results in altered production of the interleukins. These studies point to the importance of PT in altering the immune system in a manner which permits the continuance of infection. Studies aimed at elucidation of the regulating pathways, affected by pertussis toxin action, may provide greater insight into the mechanisms which modulate immune response. More detailed description of the role of PT in modulating CTL production is discussed in project Z01 HD 00073-15.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 HD 01304-04 LDMI
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)</b> Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</b>		
PI:	John B. Robbins	Head LDMI, NICHD
Others:	Shousun Chen Szu	Senior Staff Fellow LDMI, NICHD
	Rachel Schneerson	Senior Investigator LDMI, NICHD
	Tod Cramton	Guest Researcher LDMI, NICHD
<b>COOPERATING UNITS (if any)</b> H. Kornhof, South African Institute of Medical Research; I.L. Acharya, Infectious Disease Hospital, Kathmandu, Nepal; R. Kumar, All India Institute of Medical Sciences; C.U. Lowe, OD, NICHD; M. Cadoz, Institut Merieux, Lyon, France		
<b>LAB/BRANCH</b> Laboratory of Developmental and Molecular Immunity		
<b>SECTION</b> Section on Bacterial Disease Pathogenesis and Immunity		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL 0.3	OTHER: 1.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> <p>           Typhoid fever (TF) remains a serious and frequent cause of morbidity and mortality in underdeveloped nations; there is yet, no satisfactory vaccine for its prevention. The immunopathogenic role of the capsular polysaccharide of <u>Salmonella typhi</u> (Vi antigen), the causative agent, remains controversial. There is indirect evidence that Vi antibodies could confer protection against typhoid fever. TF is a disease of humans only; clinical studies are required to evaluate the effectiveness of the Vi vaccines. In collaboration with Dr. H. Kornhof, South African Institute of Medical Research, Dr. I. L. Acharya, Infectious Disease Hospital, Kathmandu, Nepal, and Dr. Ramesh Kumar, All India Institute of Medical Sciences, New Delhi, India, we are studying typhoid fever and Vi antibodies. An accurate, sensitive radioimmunoassay for Vi antibodies has been established; it is a reliable method to identify carriers. Studies in U.S. armed forces recruits showed the Vi elicited higher levels of Vi antibodies than cellular typhoid vaccine. Three surveys in individuals of various ages in Chile, Eastern Transvaal, and Nepal, showed that adults had considerably higher Vi antibodies than those in the U.S. Placental transmission of Vi antibodies was poor. There is an age-related development of Vi antibodies; the highest levels here were in young adults. Vi polysaccharide with low LPS content and high molecular weight prepared by the Institut Merieux, was studied for its safety and immunogenicity in Eastern Transvaal and in Nepal. The Vi elicited no serious side reactions and both 25 and 50 micrograms stimulated a 4-fold or greater response in at least 90 percent of vaccinates. Effectiveness trials are now underway. A Vi-cholera toxin conjugate has been prepared and standardized; it is about 15 times more immunogenic in mice than the Vi alone. Clinical studies with this new vaccine are being planned.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01306-03 LDMI

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pertussis Heat Labile Toxin (HLT): Isolation and Characterization

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald Sekura Research Chemist LDMI, NICHD

Others: Yan-ling Zhang Visiting Associate LDMI, NICHD  
Robin Roberson Chemist LDMI, NICHD  
Xiuru Li Visiting Fellow LDMI, NICHD

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Bacterial Disease and Pathogenesis and Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL:

2.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bordetella pertussis produces several protein toxins including pertussis toxin (see project Z01 HD 1302) and dermonecrotic toxin or heat labile toxin (HLT). HLT, when injected subcutaneously into suckling mice, results in a local pronounced hemorrhagic lesion. The extent, localization, and nature of this toxin-induced injury suggests HLT contributes to the pathogenesis of B. pertussis. Conditions for assay of the toxin, using the suckling mouse model, have been established. Homogeneous preparations of HLT have been obtained and it has been established that the toxin is a single polypeptide chain with a molecular weight of about 150,000.

The purified toxin has been used to produce antibodies. These antibodies have been used to document the purity of current toxin preparation. In addition, they neutralize toxic activity and high titer sera (1/20,000) have been obtained from immunized rabbits. Passive immunization of mice with HLT directed antibody protects mice against challenge with live B. pertussis suggesting that HLT may serve as a protective antigen. To investigate this possibility in greater detail, monoclonal antibodies to HLT are being produced.

The toxicity of HLT is poorly understood and more detailed information establishing its role in pathogenesis is needed. Preliminary studies in which purified HLT was injected into mice indicate that the effects of the toxin are broad and histologic changes affect tissues such as spleen, liver, marrow, and thymus. Understanding of the pathologic changes in these tissues should contribute to elucidation of its mechanism of action and role in disease.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01307-03 LDMI
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pertussis Toxin: An Approach to a New Pertussis Vaccine		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ronald Sekura	Research Chemist LDMI, NICHD
Others:	Yan-ling Zhang	Visiting Associate LDMI, NICHD
	Nathaniel Tolson	Biologist LDMI, NICHD
	Birger Trollfors	Visiting Fellow LDMI, NICHD
	Brett Acton	Biologist LDMI, NICHD
	Robin Roberson	Chemist LDMI, NICHD
COOPERATING UNITS (if any)  J. Shiloach and B. Kaufman, NIDDK		
LAB/BRANCH Laboratory of Developmental and Molecular Immunity		
SECTION Section on Bacterial Disease Pathogenesis and Immunity		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
2.67	1.07	1.6
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided ) <p>             The incidence of pertussis has been controlled by use of our current whole cell pertussis vaccines. Recent advances in identification of pertussis toxin (PT) as a major protective antigen against pertussis affords an opportunity to produce a new vaccine with improved safety and efficacy. The current project concentrates on development of methods for production of PT, as well as methods for neutralization of toxic action and assessing the immune response to the resultant pertussis toxoid vaccine. Growth conditions and purification methods have been developed to produce PT in the quantities necessary for clinical study. Assay methods for monitoring the toxicity and neutralization of toxicity of experimental vaccine preparation have been established. In addition, methods have been established to monitor the retained antigenicity of toxoided PT preparations, and to monitor immune response to PT-toxoids in mice, monkeys, and humans, and also to measure vaccine efficacy in mice and tissue culture. Methods have developed to inactivate pertussis toxin and prepare pertussis toxoids. Pertussis toxoids, prepared in our laboratory, show only minimal levels of residual toxic activities, while antigenic activity is only partially reduced. Experimental vaccines prepared by absorption of pertussis toxoid onto aluminum hydroxide are highly antigenic and elicit a PT specific immune response. Immunization with this pertussis toxoid protects mice against both bacterial and toxin challenge and elicits serum antibodies, which neutralizes PT in the CHO cell assay. Immunization of juvenile Rhesus monkeys with the vaccine leads to a similar increase in PT neutralizing antibodies. On the basis of the safety and immunogenicity in animals, a protocol for clinical investigation of the vaccine has been drafted and approved by the FDA and NIH. Clinical evaluation of the new pertussis toxoid vaccine in humans is now beginning.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01308-03 LDMI

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Conjugation of Pneumococcal and Vi Polysaccharides with Carrier Proteins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Shousun Chen Szu Senior Staff Fellow LDMI, NICHD

Others: John B. Robbins Head LDMI, NICHD

## COOPERATING UNITS (if any)

John L. Inman, Laboratory of Immunology, NIAID

## LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

## SECTION

Section on Bacterial Disease and Pathogenesis and Immunity

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

1.3

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Heterobifunctional reagent, N-succinimidyl 3-(2-pyridylthio) propionate (SPDP) was used for synthesis of polysaccharide and protein conjugates that could be considered for human use. Phosphocholine (PC) specific antibodies elicited by unencapsulated pneumococci confer protection against encapsulated pneumococci. The cell wall polysaccharide (Cw-Ps) is the most likely structure reacting with these PC antibodies. The Cw-Ps was conjugated to bovine serum albumin using SPDP. Rabbits immunized with the conjugate synthesized antibodies toward the Cw-Ps backbone; no PC antibodies were detected. The rabbit antiserum did not confer protection against pneumococci types 3 and 6A in mouse model. Species-specific protection conferred by other PC binding antibodies could not be absorbed with Cw-Ps. Other cell surface PC-containing antigens will be sought using antiserum specific to Cw-Ps to remove this structure.

The Vi capsular polysaccharide (ViCPS) is a potential protective antigen of Salmonella typhi, the causative agent of typhoid fever. ViCPS was thiolated with cystamine and then conjugated with a carrier protein through SPDP. Mice immunized with ViCPS conjugate elicited five to ten times higher antibody level than with ViCPS alone; a booster effect was also observed with the conjugate. A clinical study in adult volunteers using cholera toxin or diphtheria toxoid as a carrier protein is planned.

The final product using SPDP contains a disulfide bond. Other cross-linking reagents of maleimide or a halogen will be studied for their ability to form a conjugate without a disulfide bond.



PROJECT NUMBER  
Z01 HD 01309-03 LDMI

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Bacterial Polysaccharides Cross-Reactive with Meningococcal Group A Polysaccharide

PI: Rachel Schneerson Senior Investigator LDMI, NICHD

W. Egan, OoB, FDA, A. Bax, LCP, NIDDK, E. Kabat, Columbia University, NYC,  
E.C. Gotschlich, The Rockefeller University, NYC, Ida & Frits Ørskov, Statens  
Seruminstitut, Copenhagen

Laboratory of Developmental and Molecular Immunity

Section on Bacterial Disease Pathogenesis and Immunity

NICHHD, NIH, Bethesda, Maryland 20892

0

☐ (a) Human subjects  
☐ (a1) Minors  
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK (use standard unredacted type. Do not exceed the space provided.)

Similar to other encapsulated bacterial pathogens, anti-capsular polysaccharide antibodies confer immunity to diseases caused by Neisseria meningitidis. Group A meningococcal diseases have a different epidemiology than the other pathogenic serogroups. In Africa, Group A meningitis is endemic with high frequency; in other parts of the world, Group A causes epidemics. In both situations, asymptomatic carriage of Group A meningococci is low. In the U.S., disease or carriage of Group A meningococci can be considered as absent for the past 30 years. Yet, most children and young adults throughout the world have Group A polysaccharide antibodies. During investigations to find cross-reactive polysaccharides among non-pathogenic normal flora that might account for this ubiquitous "natural" immunity, two Escherichia coli capsular polysaccharides, K93 and K51, were discovered and their serological properties and structures characterized. Despite their antigenic cross-reactivity, the K93 and K51 failed to absorb Group A antibodies from pre and post immunization sera of vaccinates injected with Group A meningococcal vaccine. Importance of the O-acetyl in the K93 polysaccharide in this cross-reactivity was established. Schemes for synthesis of K93, K51 and Group A polysaccharides with medically useful carrier proteins have been devised in order to prepare conjugates for clinical evaluation. Such products should be more effective immunogens against Group A meningitis in infants and children than Group A vaccine.

GPO 914-918

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

- Z01 HD 00047-17 Biochemical Studies of Neuronal and Other Cell Types  
Douglas E. Brenneman, Ph.D.
- Z01 HD 00048-12 Studies of Transcriptional Control of Neurobiologic  
and Development Phenomena  
Bruce K. Schrier, M.D., Ph.D.
- Z01 HD 00064-10 Neurobiologic Studies of Neurons and Glia in Cell Culture  
Phillip G. Nelson, M.D., Ph.D.
- Z01 HD 00094-16 Pineal Regulation: Environmental and Physiological  
Factors  
David C. Klein, Ph.D.
- Z01 HD 00095-16 Pineal Regulation: Transsynaptic and Intracellular  
Mechanisms  
David C. Klein, Ph.D.
- Z01 HD 00704-03 Tetanus Toxin Effects and Localization in Neurons  
(Inactive)
- Z01 HD 00706-02 Physiological Studies of Nervous System Development  
In Vitro  
Gary L. Westbrook, M.D.
- Z01 HD 00707-02 Pharmacological Studies of Synaptic Transmission  
In Vitro  
Mark L. Mayer, Ph.D.
- Z01 HD 00708-02 Morphologic Studies of Neuronal and Non-Neuronal Cells  
in CNS Cell Cultures  
Elaine A. Neale, Ph.D.





NICHD ANNUAL REPORT  
October 1, 1985 to September 30, 1986

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

The Laboratory of Developmental Neurobiology is currently composed of the following Sections:

- 1) Section on Neurobiology headed by Dr. Phillip Nelson and including biochemical studies by Dr. Brenneman and co-workers; physiological studies by Drs. Mayer, Westbrook and colleagues, and morphological studies by Dr. Neale and colleagues. There is strong interaction between these groups.
- 2) Section on Neuroendocrinology headed by Dr. David Klein, which focuses on the physiology, pharmacology, and increasingly the molecular and cell biology of the pineal gland.
- 3) Unit on Molecular Neurobiology headed by Dr. Bruce Schrier.

Two primary interests have motivated much of the work done in the Section on Neurobiology of the LDN over the past several years. The first has been an attempt to understand the basic mechanisms responsible for excitatory synaptic transmission in the mammalian central nervous system. We have been involved with studies of both synaptic excitatory transmitter release and the postsynaptic membrane response initiated by excitatory agents. A second area of interest concerns the roles that electrical and synaptic activity may play in shaping nervous system development. The mature functional architecture of synaptic circuitry in the mammalian brain is critically dependent on the pattern of activity imposed on it during development by the structure of the environment. We have sought to understand some of the molecular and cellular processes involved in this coupling between environment stimuli and nervous system form and function. Obviously related in a general way, these two primary areas of interest during the last year or so have begun to reveal important and experimentally relevant overlap of findings.

More general questions of regulation are dealt with in the Section on Neuroendocrinology and the Unit on Molecular Neurobiology.

Dr. Klein's Section on Neuroendocrinology has taken the cells of the pineal gland as the system for analyzing in pharmacological, cell biological and molecular genetic terms the regulation of cell function that is produced by a neurotransmitter, norepinephrine. In receiving the NIH Director's award this year, Dr. Klein was cited "For pioneering studies of the pineal gland which have demonstrated the unique role of this organ in the neural regulation of metabolism and biological rhythms." He and his group have demonstrated the involvement of different adrenergic receptors and the complex interaction between these receptors as they are activated. They have developed the techniques for directly addressing the cell biologic mechanisms of altered intracellular calcium ion concentration and second messenger activation that pineal cell stimulation produces. Activities are under way directed at molecular cloning of specialized pineal genes which should allow the analysis of regu-

lation at the level of transcription, and the availability of antibodies to several gene products has resulted in immunocytochemical and quantitative study of such proteins. A major finding from this work has been that pineal cells send axonal projections into the central nervous system, so that a classically neuronal influence as well as a neurohumoral effect of the pineal on brain function may be anticipated.

The MNU has continued with efforts to isolate and characterize differentiation of specific genes, using mouse neuroblastoma cell lines. Work is continuing with a specific gene, that for choline acetyltransferase (ChAT); sequencing of a gene tentatively so identified on the basis of colony selection of an expression library with an anti-ChAT antibody has not yet unambiguously established homology between the cloned sequence and the authentic gene. Collaborative work is concerned with isolating the gene coding for a neurotrophic factor produced by brain tissue near a stab wound in the rat cerebral cortex.

### Section on Neurobiology

#### Neuron-glial-neuron peptide mediated interaction

Powerful neuron-glial interactions with major impact on nervous system development are proving amenable to detailed cell-biologic analysis in the mammalian cell culture systems employed in the Laboratory. Dr. Brenneman has shown that a neuropeptide, vasoactive intestinal peptide (VIP), is released from a subset of neurons in an activity dependent manner, and acts on non-neuronal cells, presumably glia, at concentrations as low as  $10^{-12}$  M. A large increase in the release of several radioactively labelled, newly synthesized proteins can be demonstrated from glial cultures in response to VIP application. No such response occurs with neuronal cultures. This macromolecular material released by peptidergic stimulation of non-neuronal cells essentially abolishes the substantial neuronal death that normally occurs in culture in close approximation both temporally and quantitatively to the developmental loss of neurons that occurs in vivo. Exogenously supplied VIP produces the glial response and experiments with either anti-VIP antibodies or VIP fragments that block the VIP receptor convincingly demonstrate that endogenously released VIP has a similarly potent glia-mediated effect on neuronal survival. Closely related peptides with substantial homology to VIP either lack any such effects or are active only at substantially higher concentration (plausibly working through partial agonistic activity at the VIP receptor). These findings are particularly exciting in that they may provide a generalizable model for a major aspect of activity-dependent modification of nervous system development. (It is noteworthy that the widespread distribution of VIP-containing cells in the cerebral cortex would allow this system to subserve a broad developmental role there). It seems unlikely, however, that VIP is the only agent that will prove capable of eliciting release of trophic material from glial cells and some evidence exists that catecholamines may do so. We hypothesize that a number of broadly distributed peptidergic and aminergic systems in the brain may have a specific developmental regulatory role mediated by the sort of neuron-glial-neuron interaction that we have demonstrated. The culture systems allow detailed analysis of the neuron-derived signal acting on the glia receptors, the second messenger systems, and the neurotrophic materials that are released. The possible significance for neurodevelopmental

disorders are substantial.

A developmental effect of neural electrical activity complementary to that described above is its role in producing neuronal death. A large number of elegant studies have demonstrated in vivo that electrical activity is instrumental in the loss of neurons that is so characteristic of normal development. In our culture system, also, it can be shown that neuron survival in electrically active cultures treated with VIP is less than in electrically inactive cultures also treated with VIP or conditioned medium from VIP-treated glial cultures. (Electrically inactive cultures not treated with VIP or conditioned medium have the lowest neuronal survival).

A closely related area of study has to do with specific regulation of the development of central cholinergic neurons. The activity of choline acetyltransferase (ChAT) is increased in spinal cord cultures exposed to culture medium conditioned by glial cells. We have shown, however, that activity related regulation of neuro-development is specific for different cell types. (Inhibitory neurons that take up gamma-amino-butyric acid are relatively insensitive to alterations in the state of electrical activity in the cultures). It may be that different growth factors selectively affect different types of neurons. We have begun fractionation of glial-conditioned medium and will use the convenient ChAT activity assay system for initial evaluation of the different fractionation protocols. Comparison of ChAT stimulation activity with overall neuronal survival promoting activity in selected fractions will be useful in identifying differential cell specific effects of different trophic molecules. Growth factors already identified, such as nerve growth factor, will be utilized in this regard as well. Identification of cholinergic neurons by an anti-ChAT antibody that we have used extensively will allow determination of numbers of surviving cholinergic neurons as well as the aggregate enzymatic activity of the cultures under different experimental conditions. Experiments have begun on cholinergic development in cultures of the medial septal region of the brain, one of the two major sources of cholinergic efferents to neo- and archicortex. The involvement of these systems in central information storage and in major neuro-pathologies makes direct experimental extrapolation of findings from spinal cord to these systems of high priority.

Drs. Neale and Nelson have initiated a series of experiments to address the mechanism of electrical activity related to neuronal loss and the closely related problems of synapse elimination and stabilization, using a compartmental culture system that we feel should give unique advantages for such analysis. These experiments will interdigitate closely with the studies discussed above and, using the immunocytochemical and physiologic methods with which we have experience, will be designed to test a number of specific hypotheses as to the cellular basis for the extensive synaptic remodelling and cell death that occur during development as a consequence of patterned neuronal electrical activity. Considerable methodological work will be involved in these studies, but we consider that this is fully justified by the central role that these regressive processes play in functional development of the nervous system.



### Excitatory synaptic transmitter release mechanisms

Combined physiological and anatomical studies of cultured spinal cord neurons by Drs. Neale and Nelson have been directed at the question of the morphological identity of the physiological transmitter release element. While it appeared that the synaptic bouton corresponds to the release element, in some cases up to three-fourths of the boutons may be physiologically inactive representing a functional "synaptic reserve" available for mobilization under appropriate circumstances. Inadequate regulation of intracellular calcium in some cell types or in some synaptic boutons may contribute to this functional inactivation; raised intracellular calcium ion concentration would be expected to diminish the availability of voltage-sensitive calcium channels necessary for transmitter release. We found that DRG neurons (as compared to SC neurons) were less able to maintain either transmitter output or voltage-sensitive calcium currents under conditions of sustained repetitive activation. These cells also appear to have a lower concentration of mitochondria in their synaptic boutons than do SC neurons.

Further pharmacological studies of calcium currents and transmitter release have utilized several opiate peptides and dihydropyridine agonists and antagonists of voltage-sensitive calcium channels. We were interested in the selectivity of the distribution of presynaptic receptors on tissue-cultured neurons and the question of the type of calcium channels that are responsible for transmitter release.

Delta ( $\delta$ ) receptors could be demonstrated in all tested DRG neurons and about two-thirds of SC neurons; activation of these receptors results in decreased transmitter output assayed electrophysiologically. Kappa ( $\kappa$ ) receptors occur on some one-third of SC neurons, but only in co-occurrence with  $\delta$  receptors. Mu ( $\mu$ ) receptors could be demonstrated only in a minor (<5%) proportion of the SC cells tested.

Dr. R.W. Tsien and colleagues have demonstrated three species of calcium channels of which the activation of one (the L channel) is increased by the dihydropyridine BayK 8644. We have confirmed the agonist action of this agent in cultured DRG and SC neurons and shown that BayK 8644 does not have a corresponding effect of increasing transmitter output from these cells. This suggests that L channels may not be involved in transmitter release from these cells. We have found that nitrendipine, a blocker of voltage-sensitive calcium channels in many tissues, generally does not significantly affect calcium currents in cultured SC or DRG neurons. On average, no significant effects on transmitter output could be documented, although in a minority of cells some augmentation of output occurred with nitrendipine application.

In further work in this area, we hope to establish what specific type of calcium channel is involved in transmitter release. This would have important neuropharmacological consequences, in that the different calcium channels are affected differently by different drugs.

### Excitatory amino acids; postsynaptic receptor mechanisms

Drs. Mayer and Westbrook have now provided a relatively complete picture of the functioning of the major receptor systems mediating classical excitatory

synaptic transmission in the central nervous system. A large body of heretofore paradoxical experimental results in the literature have been rationalized by the findings, widely cited in the field, of Mayer and Westbrook. At least two and probably three types of receptors have been demonstrated in experiments *in vivo*. These are termed, on the basis of agonist effectiveness, kainate, quisqualate or N-methyl-D-aspartate (NMDA) receptors.

NMDA receptors appear to be the most complex, and in a number of regards, the most interesting. The channels associated with this receptor are chemically gated but exhibit a strongly voltage-dependent behavior which is due to blockade of the channels by magnesium ions. The functional morphology of the channels has been explored by measuring their conductance at various potentials in the presence of different divalent ions. The results suggest that an ion-binding site occurs in the channel with a selective affinity for the different ions. Binding of divalent cations reduces the permeability of the channels to monovalent cations and the binding energy determines the voltage-dependent behavior of the channels. The NMDA channel has a relatively high permeability to calcium ions; the ratio of calcium to sodium permeability is approximately 5, an unusually high value for chemically gated channels. Measurements of changes in intracellular calcium with calcium-sensitive dyes has demonstrated that NMDA receptor agonists produce an increase in intracellular calcium, while activation of kainate or quisqualate receptors do not show comparable changes. Other experiments confirm this unique calcium permeability feature of the NMDA receptor-associated channels.

This calcium ingress associated with NMDA receptor activation is of particular interest in view of the involvement of changed intracellular calcium in a variety of cellular regulator processes. Thus, it is important to understand the relationship of these receptors to synaptic transmission in the central nervous system. Previous studies had implicated non-NMDA receptors in the mediation of fast excitatory synaptic transmission in most central synapses. Recent experiments in the LDN have demonstrated clearly, however, that most, if not all, spinal cord excitatory synapses involve a dual receptor mediated mechanism. Experiments utilizing manipulations of neuronal membrane potential,  $Mg^{++}$  ion concentration and selective amino acid blockers, reveal that EPSPs are composed of a fast non-NMDA receptor mediated component and a much slower component, mediated by NMDA receptors. Since activation of NMDA receptors has also been implicated in several pathological situations (epilepsy and hypoglycemic brain damage), an understanding of their role in synaptic transmission is vital to an understanding of both normal and abnormal CNS function. We have been successful in establishing tissue culture preparations where such synaptic mechanisms may be expected to be expressed as a longer term modification or plasticity of synaptic function. In particular, hippocampal neurons *in vivo* or in slice preparations exhibit long term potentiation of excitatory synaptic input under a variety of circumstances. Cultures of hippocampal neurons, in conjunction with their normal cholinergic input from the septal region, offer favorable opportunities for studying physiological mechanisms of neuronal plasticity in an extremely accessible experimental system.

The findings of Drs. Mayer and Westbrook on excitatory synaptic mechanisms have clear relevance for the activity-dependent developmental modulations being analyzed by Dr. Brenneman's group. Pharmacological tools are available



to address the question of which class of amino acid receptors may be involved in those developmental phenomena.

## Section on Neuroendocrinology

### Cyclic nucleotide regulation

Noradrenergic neural stimulation of the pineal gland provides dramatic regulation of melatonin synthesis by the gland. Both  $\alpha$ -1 and  $\beta$ -1 adrenoreceptors are involved in this regulation and considerable understanding has been achieved of the cell biological mechanisms activated via these two receptors and of the intricate interactions between them. The research program of the Section has produced much new information on how  $\alpha$ -1 and  $\beta$ -1 receptors interact to regulate cAMP and cGMP, which are directly involved in control of melatonin metabolism. Activation of the  $\beta$  adrenoreceptors is a prerequisite for stimulation of either cAMP or cGMP levels, but pure  $\beta$ -receptor stimulation has only a small effect on both cyclic nucleotides, representing less than 5% of the maximal response.  $\alpha$ -1 receptor stimulation has no effect on either cyclic nucleotide by itself, but simultaneous stimulation of both receptors results in an elevation of cAMP and cGMP more than 100-fold greater than control values. It is of interest that the peptide, vasoactive intestinal peptide (VIP), that Dr. Brennehan is studying in neuronal and glial cultures, can mimic the effects of  $\beta$ -adrenergic stimulation of pineal cells.

The Section on Neuroendocrinology has made considerable progress in elucidating the role that calcium and phospholipids play in cAMP and cGMP regulation. Activation of the  $\alpha$ -1 receptor produces a sustained increase in intracellular calcium due to an increase in net influx ingress of calcium across the surface membrane through a ligand-activated, nifedipine-insensitive channel. The  $\alpha$ -1 activation is accompanied also by an increase in calcium-dependent phospholipase C activity, which generates diacylglycerol from phospholipids. These changes in cytosolic calcium and diacylglycerol in turn result in activation of protein kinase C by translocating it from cytosol to the membrane of the pinealocyte. Protein kinase C acts probably via adenylate and guanylate cyclase and via GTP binding proteins, to increase levels of the cyclic nucleotides. An additional mechanism involving phospholipase  $A_2$  and arachidonic acid has been implicated in regulation of cGMP. Mepacrin, an inhibitor of phospholipase  $A_2$ , blocks adrenergic stimulation of cGMP.  $\alpha$ -1 receptor stimulation of phospholipase  $A_2$  may be mediated by protein kinase C, since activation of this kinase activates the phospholipase. According to these findings, then, the cascade of events leading to an increase in cGMP may first involve  $\alpha$ -1 stimulation of calcium ingress and diacylglycerol production (resulting from phospholipase C activation), followed by protein kinase C activation which stimulates phospholipase  $A_2$ . Increased activity of this enzyme results in production and release of arachidonic acid; this agent, or an active metabolite, appears to fully activate  $\beta$ -1 sensitive membrane bound guanylate cyclase. This line of work will be actively pursued and is deemed important in that relatively little information is available regarding the molecular basis for cGMP regulation in neural tissue.

Continued adrenergic stimulation of the pineal gland results in desensitization and findings in the Section indicate that this may involve protein



kinase C; activators of this enzyme not only have stimulatory effects but also produce desensitization to adrenergic agonists. Long term effects seen in pinealocytes deprived of adrenergic stimulation are also of interest to the Section. In this case a large shift in responsivity of the gland to adrenergic stimulation is seen such that cAMP responses dominate over cGMP responses.

Cellular mechanisms related to regulation of the pinealocyte membrane potential are of significance in that NE produces a membrane hyperpolarization and this hyperpolarization appears to be required for the adrenergic stimulation of N-acetyltransferase activity and of melatonin production. Ouabain or high  $K^+$  block such stimulation. Studies of an ouabain-sensitive Na-K-ATPase have shown that this enzyme is absent in pinealocytes at birth and only develops postnatally. Correspondingly, ouabain does not block the adrenergic stimulation of N-acetyltransferase activity in pinealocytes at birth, although a high concentration of  $K^+$  does. Interestingly, activation of phospholipase  $A_2$ , acting through the generation of fatty acids, appears to affect the transmembrane position of Na, K-ATPase, resulting in a 3-fold increase in the number of external, ouabain binding sites with total inhibition of internal, catalytic sites. Thus adrenergic activation of phospholipase  $A_2$  might decrease Na pump activity, depolarizing the pinealocyte.

#### Melatonin regulation

cAMP increases the production and release of melatonin by increasing the activity of one of the enzymes involved in the conversion of serotonin to melatonin. This is a two-enzyme pathway involving an N-acetyltransferase and an O-methyltransferase. N-acetyltransferase regulates the daily rhythm in melatonin synthesis and is regulated by norepinephrine acting through a cAMP mechanism. cAMP stimulates the activity of rat N-acetyltransferase 100-fold. This stimulation involves new synthesis of protein and gene expression, and is rapid, with a doubling time of about 15 minutes. cAMP also stabilizes N-acetyltransferase in an active form, preventing inactivation. When cAMP decreases, N-acetyltransferase activity decreases rapidly, with a halving time of about three minutes.

Hydroxyindole-O-methyltransferase is also regulated by norepinephrine. However, it changes gradually in response to stimulation, and changes are detected only following a week of treatment.

As indicated above, the adrenergic-cyclic AMP stimulation of N-acetyltransferase requires membrane hyperpolarization. Some recent studies have indicated that depolarization by  $K^+$  blocks gene expression, and thereby blocks the increase in N-acetyltransferase. Ouabain appears to block the increase in N-acetyltransferase at a latter step, allowing gene expression but appearing to prevent translation.

This line of investigation is of special importance because it is a rare example of a role of membrane potential in gene expression in the nervous system, and will be important to pursue.

The entire study of the regulation of N-acetyltransferase and hydroxyindole-O-methyltransferase activity in the pineal gland is important because it

will provide some insight into the adrenergic regulation of gene expression in the nervous system. Up to this time, studies have been almost entirely limited to the analysis of enzyme activity, with no meaningful measurement of enzyme protein or mRNA, or related studies on post-transcriptional and post-translational procession. These have not been possible because the appropriate probes have not been available.

The Section on Neuroendocrinology has initiated a major effort to obtain the tools needed to study the regulation of N-acetyltransferase and hydroxyindole-O-methyltransferase, and related proteins of interest.

Highly purified preparations of sheep N-acetyltransferase have been obtained in sufficient amounts to sequence the enzyme and initial steps have been taken to raise antiserum.

The bovine enzyme, hydroxyindole-O-methyltransferase, has been prepared in suitable amounts of highly pure material so that the sequence of this protein is now being determined; a partial sequence has been established. High titre polyclonal antiserum with high specificity has been prepared in rabbits, and an extensive characterization has indicated this is a reliable means of detecting the bovine enzyme.

#### cdNA probes

To prepare cdNA probes for N-acetyltransferase and hydroxyindole-O-methyltransferase, a bovine pineal cdNA library was prepared in collaboration with the Unit on Molecular Neurobiology in the LDN. An improved bovine pineal cdNA library is now being prepared, along with a day-sheep and night-sheep pineal cdNA library, and a control-rat and adrenergically-stimulated rat pineal cdNA library. These will be used to clone specific cdNA's of interest, for eventual experimental use in analysis of gene expression in the pineal gland.

In an associated effort to study the regulation of gene expression in the pineal gland, work has been done on the isolation of the S-antigen gene. The S-antigen, a putative cyclic nucleotide regulatory protein, is found only in the pineal gland and retina.

#### Additional studies

A number of observations indicate that the paraventricular nucleus of the hypothalamus may be involved in the circuits which control the release of norepinephrine from the sympathetic nerve endings in the pineal gland. This past year the Section has collaborated with workers at the University of Pennsylvania, Department of Psychology, to show that electrical stimulation of the paraventricular nucleus during the day causes an increase in melatonin production. This work is continuing with iontophoresis of putative transmitters.

In collaboration with Dr. Horst Korf, Geissen, West Germany, the Section reported this year that there are neural connections between the pineal gland and both the habenula and posterior commissure. This is a highly important discovery because it provides reason to suspect that the pineal gland might

communicate directly with other areas of the brain, not only by humoral avenues. The work on pineal projections has continued in a collaborative effort with Drs. Ungerlieter and Mishkin, NIMH, using the monkey. Preliminary findings point to the possibility that such connections also are present in primates. This work may be pivotal for future research in the pineal gland.

### Molecular Neurobiology Unit

The Molecular Neurobiology Unit has continued its work with differentiation-stage-specific cDNAs from mouse neuroblastoma cells and from a hybrid cell line (mouse neuroblastoma x rat glioma). To date, several differentiation-specific and differentiation-enriched clones have been isolated and the degree of specificity confirmed by Northern blots and/or dot blots. RNA blots are also being used to determine the environmental stimulus responsible for activation of the expression of these genes and the timing of that activation during the differentiation paradigm.

We have recently been involved with efforts to determine whether the cDNA clone designated  $\lambda$ CHAT7 is homologous to the mRNA which encodes the enzyme choline acetyltransferase. By means of in vitro translation of hybrid-selected mRNA, followed by assays for enzyme activity and Western blot identification with monoclonal and polyclonal antibodies, we are trying to obtain additional evidence for this clone's identity.

Recently, we have employed the cascade hybridization technique to partially purify cDNAs enriched in rat cerebral cortical tissue near a stab wound 10 days after the lesion. A library of these cDNAs cloned in an expression vector has been obtained, is being characterized as to its specificity for lesioned brain, and will be screened for a neurotrophic factor necessary for the survival of sympathetic neurons in culture. This factor is known to be much enhanced in lesioned brain tissue.

We have achieved the expression of mouse pro-opiomelanocortin (POMC) in *E. coli*. The 5'-untranslated region of a full-length POMC cDNA clone was exonucleolytically deleted and the coding sequence was inserted into a bacterial expression vector within which transcription of the POMC coding sequence is under the control of the lac operator. Cells that harbor the clones can be induced to synthesize large amounts of the POMC protein. POMC analogues will be produced from specifically mutagenized clones and, in collaboration with the Cellular Neurobiology Unit (LNN), we intend to participate in an investigation of the basis of the cleavage-site specificity of a pro-hormone converting enzyme via analyses of the cleavage products produced by reaction of the converting enzyme with the POMC analogues.

Primary cultures of glial cells have been observed by others in the Laboratory of Developmental Neurobiology to secrete a neuronotrophic factor that stimulates choline acetyltransferase activity in fetal mouse spinal cord neurons. We have observed that Xenopus laevis oocytes that have been injected with glial cell-derived RNA will secrete a neuronotrophic factor that exhibits the same properties as that detected in glial cell conditioned medium (mock-injected oocytes do not produce the factor). Hence we have an assay for the messenger RNA that encodes the factor and have undertaken the molecular cloning of its cDNA.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 HD 00047-17 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Studies of Neuronal and Other Cell Types

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Brenneman Staff Fellow LDN, NICHD

Others: R. Alderson Staff Fellow LDN, NICHD  
S. Fitzgerald Biologist LDN, NICHD  
D. Warren Bio-Lab Tech. LDN, NICHD  
E. Neale Physiologist LDN, NICHD

## COOPERATING UNITS (if any)

L. Eiden LCB, NIMH, J. Patel LCS, NIMH, R. Siegel LCB, NIMH; M. Litzinger, Univ. of Utah Med. Ctr, Salt Lake City, Utah.

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS

3.6

## PROFESSIONAL:

2.3

## OTHER:

1.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

Cell cultures prepared from fetal mammalian central nervous system were utilized to study the regulation of neuronal survival and the differentiation of cholinergic neurons. The interaction between glia and neuropeptides was found to be a determinant of neuronal survival during development. Vasoactive intestinal peptide (VIP) produced an increase in the survival of neurons when spontaneous electrical activity was blocked. This effect of VIP was mediated by a substance released from a non-neuronal cell type. Closely homologous peptides to VIP (PHI-27, secretin, growth hormone releasing factor) either had no such action or required very high concentrations to produce increases in neuronal survival. VIP was shown to increase the efflux of newly synthesized proteins from non-neuronal spinal cord cultures but not from cultures composed primarily of neurons. The release of proteins was stimulated by  $10^{-12}$  M VIP. The effect on protein release was attenuated at concentrations greater than  $10^{-8}$  M. This dose response relationship was similar to that observed for the increase in neuronal survival produced by VIP. An endogenous factor produced by glial-enriched spinal cord cultures was found to increase the activity of choline acetyltransferase (CAT). The trophic substance was partially purified and a more complete purification scheme developed. Total mRNA was isolated from glial-enriched cultures. Injection of the glial mRNA into frog oocytes resulted in synthesis of proteins that produced a 3-fold increase in CAT activity. Phorbol esters at 10 nM concentration produced a significant stimulation of CAT activity in spinal cord cultures while at higher concentrations they produced a reversible reduction in CAT activity. The phorbol ester effects in spinal cord cultures appear to be mediated by protein kinase C.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00048-12 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies of transcription-level control of neurobiologic &amp; developmental phenomena

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.K. Schrier Medical Officer LDN, NICHD

Others: Eugene T. Butler Special Expert LDN, NICHD  
 Massimo Pandolfo Visiting Fellow LDN, NICHD  
 Robert Glatte Bio. Lab. Aid LDN, NICHD  
 Jane Silberman Summer Intern LDN, NICHD

## COOPERATING UNITS (if any)

M. Giovanni, B. Raj-Amaladoss, M. Nirenberg, LBG, NHLBI; T. Quack, A.M. Duchemin  
 and D. Chuang, NIMH; L. Hersh, Univ. of Texas HSC at Dallas.

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Molecular Neurobiology Unit

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS

2.3

## PROFESSIONAL

2.0

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

(1) Characterization of cDNA clones selected from a library of Xenopus anterior pituitary cDNAs by the mouse POMC cDNA is continuing. The clone containing the REM1 repetitive sequence is being sequenced by use of internal primers. Another clone may contain regulatory sequences for Xenopus POMC and is expected also to contain some POMC coding sequence. (2) In the neuroblastoma project, several cDNA clones with quantitative or qualitative specificity for either differentiated or undifferentiated cells are being characterized by Northern, Southern, and dot blots, nuclease protection and primer extension experiments, and sequencing. Interesting inserts are being transferred to a riboprobe-like vector to facilitate these studies, and a genomic library from differentiated NS20Y neuroblastoma cells has been prepared. (3) The cloned cDNA  $\lambda$ ChAT7, presumed to correspond to a portion of human choline acetyltransferase has been sequenced using internal oligonucleotide primers. Efforts to determine definitively whether this cDNA represents ChAT are continuing while, in the meantime, additional clones are being sought by screening with synthetic oligonucleotide probes whose sequences have been deduced from two cyanogen bromide peptides sequenced from the human placental enzyme. (4) A new project has been initiated in which cDNA clones are being obtained which are unique to, or enriched in, the subchronic reaction around a stab wound in rat cerebral cortex. Of interest are all lesion-induced mRNAs, but particularly that for a trophic factor expressed preferentially in lesioned versus control brain, which can be detected by its capacity to promote the survival of cultured sympathetic neurons. The initial characterization of a sampling of the library showed that the inserts, although small, corresponded to brain mRNAs. Characterization of these clones, as well as a search for the trophic factor(s) and preparation of another cDNA library, are in progress. (5) The cDNA coding sequence for mouse POMC was subcloned into PUC19 expression vector, and several clones which express large amounts of this protein have been obtained.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00064-10 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiologic Studies of Neurons and Glia in Cell Culture

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.G. Nelson	Head	LDN, NICHD
Others:	F. Wang	Visiting Assoc.	LDN, NICHD
	C. Yu	Visiting Fellow	LDN, NICHD
	E.A. Neale	Physiologist	LDN, NICHD

## COOPERATING UNITS (if any)

J. Moskal, NIMH

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS:

3.9

## PROFESSIONAL:

2.9

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has become clear that several species of voltage sensitive calcium channels (VSCC) exist in different tissues and even within the same cell. The different types of these channels can be distinguished on the basis of their voltage and pharmacological sensitivities. We have addressed the question of which types of calcium channels may be involved in neurotransmitter release at central synapses. Nitrendipine, a blocker of VSCC in muscle does not affect neuronal VSCC and similarly has no detectable effect on transmitter release. BayK 8644 is a VSCC agonist increasing one type of VSCC, the L channel, in dorsal root ganglion (DRG) neurons. This agent does not increase transmitter output from either DRG or spinal cord (SC) synaptic terminals. This suggests that the L channel is not involved in transmitter release.

DRG and SC neurons and muscle cells have been grown in a three-compartment chamber tissue culture system. Selective growth of axons into chambers containing target tissue as well as synapse formation by axons growing from one chamber to another have been demonstrated.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 HD 00094-16 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pineal Regulation: Environmental and Physiological Factors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.C. Klein Head LDN, NICHD

Other: D. Sugden Visiting Associate LDN, NICHD  
V. Cena Guest Researcher LDN, NICHD  
P. Svoboda Guest Researcher LDN, NICHD  
C. Gonzalez-Garcia Guest Researcher LDN, NICHD

## COOPERATING UNITS (if any)

P. Skolnick, A. Basile, NIADDK; D. Jacobowitz, S. Markey, NIMH; J. Pierce, C. Gonzalez-Garcia, NIH/LBI; J. Yanovsky, J. Witcher, H. Adler, U. of PA; H. Korf, Justus Liebig U., Giessen; T. van Veen.

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neuroendocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.3

## PROFESSIONAL:

2.0

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the environmental and physiological regulation of the pineal gland, exclusive of transmembrane and intracellular regulatory mechanisms (see ZO1-HD 00095-16 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. It has been proposed that the SCN + pineal circuit passes through the paraventricular nucleus of the hypothalamus (PVN). This past year work was completed which supports this with the demonstration that electrical stimulation of the PVN stimulates the production of melatonin at a near physiological rate. In other studies, the photoneural regulation of pineal rhodopsin kinase and phospholipase C have been studied; and the developmental appearance of both phospholipase C and Na<sup>+</sup>/K<sup>+</sup>-ATPase has been examined. It has been discovered that Na<sup>+</sup>/K<sup>+</sup>-ATPase develops after birth, as indicated by both ouabain binding and ATPase activity. This indicates that another mechanism might generate membrane potential before this time.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 HD 00095-16 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Klein	Head	LDN, NICHD
Other:	D. Sugden	Visiting Associate	LDN, NICHD
	A.L. Sugden	Visiting Fellow	LDN, NICHD
	A.K. Ho	Visiting Fellow	LDN, NICHD
	R. Dubbels	Guest Researcher	LDN, NICHD
	V. Cena	Guest Researcher	LDN, NICHD
	C. Craft	Guest Researcher	LDN, NICHD
	J. Weller	Chemist	LDN, NICHD

## COOPERATING UNITS (if any)

A. Spiegel, K. Kirk, S. Beckner, NIADDK; W. Anderson, T.P. Thomas, NCI; J. Pierce, NIHLBI; M.A.A. Namboodiri, Georgetown U.; D. Goldman, C. Merrill, D. Jacobowitz, L. Ungerleider, M. Mishkin, NIMH; H. Korf, Justus Liebig U., Giessen; I. Gery, T. Shinohara, NEI.

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neuroendocrinology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6.7

## PROFESSIONAL:

5.5

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to discover the molecular basis of neurochemical transduction mechanisms, using the pineal gland as a model. This program has yielded new information on how  $\alpha_1$ -adrenoceptors participate with  $\beta_1$ -adrenoceptors in the synergistic regulation of cAMP and cGMP.  $\alpha_1$ -Adrenoceptors appear to open a ligand dependent calcium channel, which leads to a 5-fold increase in the apparent intracellular concentration of calcium. In addition  $\alpha_1$ -adrenoceptors alter phospholipid metabolism, by stimulating the activity of both phospholipase A<sub>2</sub> and phospholipase C. The combined effects of the increase in calcium and phospholipase C activity potentiates the  $\beta$ -adrenergic stimulation of adenylate cyclase; the combined effects of the increase in calcium and of phospholipase A lead to an increase in the  $\beta$ -adrenergic stimulation of cGMP. Advances have been made in the purification of pineal N-acetyltransferase and hydroxyindole-O-methyltransferase, in the preparations of antisera against hydroxyindole-O-methyltransferase, preparation of bovine pineal cDNA libraries, cloning the S-antigen from a bovine retina cDNA library. Pineal processes have been described in a species of hamster and preliminary evidence of such processes in the monkey have been obtained.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 HD 00704-03 LDN
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Tetanus Toxin Effects and Localization in Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Neurobiology		
SECTION Section on Neurobiology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland		
TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Inactive.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00706-02 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Studies of Nervous System Development In Vitro

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Gary L. Westbrook	Staff Fellow	LDN, NICHD
	P.G. Nelson	Head	LDN, NICHD
Others:	C. L. Mitchell	Guest Scientist	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

## COOPERATING UNITS (if any)

A. B. MacDermott, LNP, NINCDS

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long term goal of this project is to study the development and regulation of electrical activity and synapse formation using dissociated mammalian central neurons in cell culture as a model system. Previous studies of these fundamental neurobiological questions have used preparations from the peripheral nervous system, i.e. sensory and autonomic ganglion cells or the neuromuscular junction. Our approach has been to use spinal cord neurons taken from mouse embryos at an early developmental stage (day 13) and use the subsequent changes that occur with time in culture as a model of development. Studies have shown that early in development, spinal cord neurons have voltage-dependent sodium, potassium and calcium channels. Recent studies have examined the chemosensitivity of these developing neurons to excitatory amino acids, which are likely to be major excitatory transmitters in this system. For both voltage-dependent sodium channels and for ion channels gated by excitatory amino acids, the major change observed during the initial 21 days in culture was an increase in receptor/channel number, whereas no change in the basic features of the ion channels (voltage-dependence and ion selectivity) was observed. In the case of sodium channels, the increase in channel number correlates with the period of synapse formation and development of spontaneous electrical activity; this suggests that regulation of ion channel density may be related to electrical activity. However, in preliminary studies we have been unable to demonstrate a similar correlation of amplitude of response to excitatory amino acids with synapse formation.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00707-02 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Synaptic Transmission In Vitro

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. L. Mayer	Visiting Associate	LDN, NICHD
	G. Westbrook	Staff Fellow	LDN, NICHD

Others:	P.G. Nelson	Head	LDN, NICHD
	I.D. Forsythe	Visiting Fellow	LDN, NICHD

## COOPERATING UNITS (if any)

A.B. MacDermott, NINCDS; M.A.A. Mamboodiri, J.H. Neale, Georgetown University;  
S.J. Smith, Yale University

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

## TOTAL MAN-YEARS:

4.2

## PROFESSIONAL:

3.7

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This goal of this project is to characterize the function of excitatory amino acids as synaptic transmitters in the vertebrate central nervous system using biophysical, physiological and pharmacological techniques for experiments on dissociated cultures of mouse CNS. Pharmacological experiments in vivo suggest separate receptors selectively activated by kainate, quisqualate and N-methyl-D-aspartate (NMDA). Micromolar concentrations of magnesium ions cause a voltage-dependent block of ion channels linked to NMDA, but not kainate or quisqualate receptors. The block by  $Mg^{++}$  shows relief on extreme hyperpolarization. At mM concentrations calcium also produces a partial block of inward current flow through NMDA receptor ion channels. The blocking action of  $Mg^{++}$  is reduced by  $Ca^{++}$ , especially on extreme hyperpolarization, suggesting competition between  $Ca^{++}$  and  $Mg^{++}$  for a site within the ion channel. One interpretation of this would be to suggest that  $Ca^{++}$  ions have a significant permeability through NMDA receptor ion channels. Reversal potential measurements on changing extracellular  $Ca^{++}$  confirm this. Measurement of intracellular  $Ca^{++}$  with the dye, arsenazo III, also shows significant calcium influx through NMDA but not kainate or quisqualate receptor ion channels. In addition to amino acids, small peptides are attracting attention as transmitter candidates. N-acetylasparylglutamate (NAAG) has been proposed as a transmitter in the olfactory cortex. In our experiments, based on dose response curves, pharmacological antagonism, and noise analysis, NAAG behaved as a weak but selective agonist at NMDA receptors and is unlikely to act as a synaptic transmitter. Synaptic potentials mediated by activation of NMDA receptors have been studied in spinal cord cultures. The NMDA receptor mediated component of the epsp is of long duration and low amplitude, but has the same latency as the epsp mediated by quisqualate or kainate receptors. These experiments suggest that a dual receptor mechanism with distinct conductances may underlie neurotransmission at synapse using excitatory amino acids as transmitters.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00708-02 LDN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphologic Studies of Neuronal and Non-neuronal Cells in CNS Cell Cultures

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Elaine A. Neale Physiologist LDN, NICHD

Others: P. G. Nelson Head LDN, NICHD  
L. M. Bowers Biologist LDN, NICHD

## COOPERATING UNITS (if any)

W. H. Habig, DBP, BB, FDA; G. K. Bergey, Univ. of Maryland, Baltimore; P. K. Sher, Univ. of Minnesota, Minneapolis; L. B. Hersh, Univ. of Texas, Dallas.

## LAB/BRANCH

Laboratory of Developmental Neurobiology

## SECTION

Section on Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL:

1.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Morphologic techniques are applied to dissociated cell cultures of central nervous system tissue. Combined electrophysiology and immunohistochemistry for choline acetyltransferase (ChAT) have provided a functional correlate for ChAT staining. Septal cultures contain a relatively large proportion of ChAT-positive neurons, hippocampal cultures contain none, and spinal cord cultures plated on feeder layers appear enriched for ChAT-positive neurons. Culture preparations are characterized routinely by immunohistochemistry for neuron specific enolase, glial fibrillary acidic protein, and fibronectin as a means of identifying neurons, astroglia, and fibroblasts, respectively. Radioautography is used to localize benzodiazepine binding sites in cerebral cortical cell cultures, and to follow the distribution, in spinal cord cell cultures, of tetanus toxin with time after initial exposure. Retrograde and anterograde axonal transport of various markers have been used to label specific cell bodies or neurites of dorsal root ganglion neurons grown in multicompartment culture chambers.



LABORATORY OF DEVELOPMENTAL PHARMACOLOGY

- Z01 HD 00136-18    Pharmacogenetics  
                    Daniel W. Nebert, M.D.
- Z01 HD 00137-12    Genetic Regulation of Drug-Conjugating Enzymes  
                    Ida S. Owens, Ph.D.
- Z01 HD 00500-08    Receptor Structure and Function  
                    Howard J. Eisen, M.D.
- Z01 HD 00503-02    Regulation and Expression of the UDP Glucuronosyl-  
                    transferase Gene Family  
                    Peter I. Mackenzie, Ph.D.





NICHD Annual Report  
October 1, 1985 through September 30, 1986

Laboratory of Developmental Pharmacology

The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular mechanisms of gene expression involving drug-metabolizing enzymes. The clinical discipline involving the study of genetic differences in drug metabolism has been termed pharmacogenetics. Endogenous (constitutive) enzymes that metabolize steroids, fatty acids, prostaglandins, leukotrienes, pheromones, thyroxine and biogenic amines also metabolize the innumerable foreign compounds that enter our body. Hundreds of drugs and other chemicals are known to stimulate (induce) their own metabolism or the metabolic fate of structurally-related compounds. In addition, steroids, prostaglandins, and small peptide hormones have been found to regulate some of these activities. The mechanisms surrounding the induction of these enzymes and expression of these genes are of central importance to fundamental molecular genetics, developmental biology, teratogenesis, carcinogenesis, mutagenesis, endocrinology, limnology, and drug addiction, tolerance and toxicity. This Laboratory presently comprises three Sections and one Unit.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., is interested in the regulation and expression of genes encoding "Phase I" drug-metabolizing enzymes, most of which represent the P450 proteins, and certain "Phase II" drug-metabolizing enzymes. The P450 gene superfamily is presently known to comprise at least eight gene families, and a protein from any of these eight families is no more than 36% similar to a protein in any of the other seven families.

Genes of the P450I family are inducible by combustion products such as benzpyrene and tetrachlorodibenzo-p-dioxin (TCDD, in the lay press called "dioxin"). The P450II gene family is the major one, with at least five subfamilies (each about 50% similar to one another) having diverged from one another about 300-600 million years ago. Genes in the IIB and IIE subfamilies are phenobarbital- and ethanol-inducible, respectively; genes in the IIA, IIC and IID subfamilies are expressed constitutively. Genes in the P450III and P450IV families are steroid- and clofibrate-inducible, respectively. Genes in the XI and XII families encode mitochondrial proteins for steroid 11 $\beta$ -hydroxylation and cholesterol side-chain cleavage, respectively. Genes in the XVII and XXI families encode enzymes for steroid 17  $\alpha$ -hydroxylation and 21-hydroxylation, respectively.

This Section is rigorously studying gene expression of the [Ah] gene battery, a group of five or more genes that are (i) under aromatic hydrocarbon (Ah) receptor regulation and (ii) sometimes activated during embryogenesis (differentiation) and tumorigenesis (dedifferentiation). The genes, which include P<sub>1</sub>450 and P<sub>3</sub>450 (the two members of the P450I gene family), NAD(P)H:menadione oxidoreductase (NMOR<sub>1</sub>), glutathione transferase (GT<sub>1</sub>) and UDP glucuronosyl-transferase (UDPGT<sub>1</sub>), have all been cloned and are being characterized. There are striking differences in transcriptional regulatory mechanisms for activation of the P<sub>1</sub>450 and P<sub>3</sub>450 genes, as well as marked developmental and tissue-specific differences in gene expression. Upstream P<sub>1</sub>450 regulatory sequences include (i) the TATA box, (ii) a TCDD-responsive enhancer (about 1000 bp upstream from the mRNA cap site) that spans more than 200 bp and includes

one or more enhancers of constitutive gene expression, and (iii) a negative control element (between 400 and 800 bp upstream from cap site) that participates in (iv) a negative autoregulatory loop. It appears that a repressor of constitutive P<sub>1</sub>450 gene transcription requires activation by a P<sub>1</sub>450-mediated metabolite and that this repressor regulates constitutive transcription of the NMOR<sub>1</sub>, GT<sub>1</sub> and UDPGT<sub>1</sub> genes as well. Genes for the Ah receptor and the putative repressor are being cloned and characterized.

Projects in this Section are divided among (1) fundamental molecular biology and genetics, (2) evolution of these genes and regulatory regions, including chromosomal walking and mapping, and (3) clinically important applications. Experimental systems include the use of inbred mouse strains, transgenic mice, recombinant DNA technology, and somatic cell genetics in culture. As an example of a clinically important application, the human P<sub>1</sub>450 and P<sub>3</sub>450 genes and flanking regions have been cloned and sequenced, and localized near the MPI gene on chromosome 15. Evidence has been presented to suggest that human P<sub>1</sub>450 and P<sub>3</sub>450 genes, similar to their orthologues in laboratory animals, are important in the activation of inert chemical procarcinogens, promutagens and proteratogens to active metabolites. Restriction fragment length polymorphisms (RFLPs) have been found, and families with high and low cancer incidence are being studied. In the future it should be possible to correlate RFLP patterns of these genes with human disease. Such tests would facilitate the evaluation of cancer and toxicity risk for individuals exposed to foreign chemicals. These assays would aid the individual, employer and physician in decisions regarding life style, cigarette smoking, employment, and prescription drugs.

B. The Section on Regulation of Gene Expression, under the supervision of Howard J. Eisen, M.D., compares the mechanism of action of the glucocorticoid receptor and the Ah receptor. Recently, it has been appreciated the glucocorticoid and other steroid hormone receptors comprise a supergene family which includes the erb-A oncogene. Since the Ah receptor also binds hydrophobic ligands and interacts with DNA, it is possible that the Ah receptor is a member of this gene family.

This Section has focused on the development of immunochemical and recombinant DNA probes for isolating and characterizing the Ah receptor protein and gene. Like steroid receptors, the Ah receptor is present at extremely low concentrations in cells; nonetheless, this Section has attempted to produce polyclonal antisera to receptor preparations which have been purified by affinity chromatography and HPLC methods. Interestingly, the Ah receptor copurifies with a group of mouse "heat shock" proteins which have the capacity also to bind hydrophobic ligands such as free fatty acids. Further studies of the structural and functional relationship of the Ah receptor to these heat shock proteins are in progress. Identification of the cDNA encoding the Ah receptor will require demonstration of the expressed functional gene product. Accordingly, in collaboration with the Section on Pharmacogenetics and Molecular Teratology, expression vectors in the yeast S.cerevisiae have been developed. cDNAs for P450 proteins, as well as chimeric P450 cDNAs, are expressed at high levels in this system. It is believed that this yeast expression system will be useful for the penultimate characterization of the Ah receptor cDNA.

In collaboration with Dr. S. Simons, the affinity-labeling steroid [<sup>3</sup>H]dexamethasone 21-mesylate has been found to bind covalently to a single cysteine residue near the carboxy terminus of the glucocorticoid receptor. These data



identify rigorously for the first time the ligand-binding "pocket" of a steroid hormone receptor.

C. The Unit on Recombinant DNA and the Conjugating Enzymes, under the direction of Peter I. Mackenzie, Ph.D., studies the regulation and expression of several subfamilies of the rat UDP glucuronosyltransferase (UDPGT) gene family. The function of the UDPGT enzymes is to conjugate oxygenated (or N- or S-containing) metabolites with glucuronic acid, thereby rendering the glucuronide conjugate extremely hydrophilic and, hence, detoxified and readily excreted. It follows logically that, if P450 oxygenates a hydrophobic drug or other chemical (Phase I metabolism) and UDPGT conjugates the oxygenated intermediate (Phase II metabolism), the two gene systems might be under some sort of coordinate regulation. Interestingly, UDPGT enzymes are similar to P450 enzymes in that (i) some genes are expressed constitutively and (ii) others are inducible by (a) combustion products such as benzpyrene and TCDD, (b) phenobarbital, (c) steroids or (d) clofibrate peroxisome proliferators such as clofibrate.

The isolation and sequencing of seven cDNA clones has demonstrated the existence of at least four different forms of transferase belonging to two gene subfamilies. One clone, pUDPGT<sub>r</sub>-2, encodes a phenobarbital-inducible form of transferase which glucuronidates 4-methylumbelliferone and the 17-OH position of testosterone and dihydrotestosterone; this was demonstrated by transfection of an expression vector containing the rat cDNA into monkey kidney fibroblast COS cells. This technique also demonstrated that a second clone, pUDPGT<sub>r</sub>-4, whose mRNA is not induced by phenobarbital or 3-methylcholanthrene, encodes a form of transferase that glucuronidates the 3-OH position of androsterone and etiocholanolone. The substrate specificity of other transferase forms are currently under investigation. Sequence studies have also shown that the transferases encoded by the three cDNAs contain signal peptide and membrane-anchoring regions and potential asparagine-linked glycosylation sites. In vitro translation studies indicate that the signal sequence is cleaved during insertion into the endoplasmic reticulum and glycosylation occurs subsequently. Genomic clones to UDPGT<sub>r</sub>-4 have been isolated and are being sequenced. Genomic and cDNA clones will be utilized to study the regulation of each form as a function of age, tissue distribution and administration of prototypic inducers.

D. The Section on Drug Biotransformation, under the direction of Ida S. Owens, Ph.D., examines the UDP glucuronosyltransferase gene family in the mouse and human. One cDNA clone has been isolated by screening a mouse cDNA-containing  $\lambda$ gt11 expression library with Dr. Mackenzie's rat cDNA clone UDPGT<sub>r</sub>-2. By sequence analysis this clone, UDPGT<sub>m</sub>-1, encodes the complete protein sequence (530 amino acids) of a mouse cDNA that is highly homologous to UDPGT<sub>r</sub>-2 and hybridizes with two phenobarbital-inducible mRNAs (2.0 and 2.2 kb) that are the result of alternative polyadenylation splicing sites. Both of these mRNAs are also induced by clofibrate (as is bilirubin UDP glucuronosyltransferase). UDPGT<sub>m</sub>-1 was used to screen a human cDNA library; a human cDNA clone (UDPGT<sub>h</sub>-1), which hybridizes to a mRNA of 2.5 kb, was isolated and is being sequenced. From a 3-methylcholanthrene-induced mouse cDNA-containing  $\lambda$ gt11 expression library, an antibody to 3-methylcholanthrene-induced UDPGT was used to isolate UDPGT<sub>m</sub>-2. This mouse cDNA insert is 2.4 kb and appears to be regulated by the Ah receptor.

Because of clinical diseases of defective bilirubin conjugation, UDPGT<sub>h</sub>-1 may prove to be a valuable probe for subsequent studies in genetic screening and

diagnosis. If UDPGT<sub>m</sub>-2 is a member of the [Ah] gene battery, this gene may be important in fundamental molecular biologic studies of gene expression, as well as projects involving detoxification of chemical carcinogens, mutagens and teratogens.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00136-18 LDP

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

PHARMACOGENETICS

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. W. Nebert Head LDP, NICHD

Others: See ATTACHMENT I

## COOPERATING UNITS (if any)

See ATTACHMENT II

## LAB/BRANCH

Laboratory of Developmental Pharmacology

## SECTION

Section on Pharmacogenetics and Molecular Teratology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS.

7.25

## PROFESSIONAL:

5.00

## OTHER:

2.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cytochrome P450 gene superfamily comprises at least eight gene families: (i) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; in the lay press called "dioxin")-inducible; (ii) phenobarbital-inducible; (iii) steroid-inducible; (iv) clofibrate-inducible; and gene(s) involved in (v) steroid 11 $\beta$ -hydroxylation; (vi) cholesterol side-chain cleavage; (vii) steroid 17 $\alpha$ -hydroxylation; and (viii) steroid C21-hydroxylation. This laboratory has studied most intensively the TCDD-inducible P450 gene family, which has two members, P<sub>1</sub>450 and P<sub>3</sub>450. In mouse P<sub>1</sub>450 and P<sub>3</sub>450 are but two genes in the [Ah] complex, a "battery" of at least five genes activated by polycyclic aromatic inducers such as TCDD and regulated by the aromatic hydrocarbon (Ah) receptor. Many of these proteins are being purified, antibodies developed, and cDNA and genomic clones isolated and sequenced in order to understand regulatory expression of this gene battery believed to play an important role early in development. There are interesting differential transcriptional regulatory mechanisms for activation of the P<sub>1</sub>450 and P<sub>3</sub>450 genes, as well as striking developmental and tissue-specific differences in gene expression. Upstream P<sub>1</sub>450 regulatory sequences include a promoter region, a negative control element (involved in a negative autoregulatory loop), and a TCDD-responsive enhancer element (about 1,000 bases upstream from the mRNA cap site) that spans more than 200 bp and includes one or more enhancers of constitutive gene expression as well. Restriction fragment length polymorphisms have been found with both the human P<sub>1</sub>450 and P<sub>3</sub>450 genes on chromosome 15. One long-range goal of this laboratory is to develop assays, based on recombinant DNA technology, to assess the human Ah phenotype and other pharmacogenetic disorders. Such assays may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.



## ATTACHMENT I - Others:

Cynthia A. Edwards	Guest Researcher	LDP	NICHD
Jacques E. Gielen	Guest Researcher	LDP	NICHD
Hana S. Haleem	Chemist	LDP	NICHD
Larry L. Heilmann	Staff Fellow	LDP	NICHD
Kiyoko Ikeya	Visiting Fellow	LDP	NICHD
Anil K. Jaiswal	Visiting Associate	LDP	NICHD
John E. Jones	Guest Researcher	LDP	NICHD
Shioko Kimura	Visiting Fellow	LDP	NICHD
Kristi L. Kotz	Federal Junior Fellow	LDP	NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP	NICHD
Roland A. Owens	Guest Researcher	LDP	NICHD
John A. Robertson	Visiting Fellow	LDP	NICHD

## ATTACHMENT II - COOPERATING UNITS:

- K. Berg, Institute of Medical Genetics, University of Oslo, Blindern, Oslo, Norway
- A. P. Bollon, Department of Molecular Genetics, The Cancer Center at Wadley Institutes, 9000 Harry Hines Blvd., Dallas, Texas 75235
- A. C. Collins, Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado 80309
- H. J. Eisen, Section on Regulation of Gene Expression, Laboratory of Developmental Pharmacology, NICHD, NIH, Bethesda, Maryland 20892
- J. E. Gielen, Laboratoire de Chimie Medicale, Institut de Pathologie, Unite de Biochimie, University of Liege, Belgium
- F. J. Gonzalez, Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20892
- J. B. Grieg, Medical Research Council, MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, England
- J.-L. Guenet, Institut Pasteur, 28, Rue Du Dr Roux, 75724 Paris Cedex 15, France
- O. Hankinson, Department of Pathology, Laboratory of Biomedical & Environmental Sciences, UCLA, 900 Veteran Avenue, Los Angeles, California 90024
- M. Kanehisa, Institute for Chemical Research, Kyoto University, Uji, Kyoto, 611 Japan
- A. Konopka, Laboratory of Mathematical Biology, National Cancer Institute, Frederick, Maryland 21701
- R. E. Kouri, IBI, P.O. Box 9558, New Haven, Connecticut 06535
- C. Kozak, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland 20205
- D. J. Lipman, Mathematical Research Branch, NIADK, NIH, Bethesda, Maryland 20892
- M. Maines, Department of Radiation Biology & Biophysics, Division of Toxicology, University of Rochester School of Medicine, Rochester, New York 14642
- O. W. McBride, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20892
- U. Meyer, Department of Pharmacology, Biozentrum, Basel, Switzerland

- I. S. Owens, Section on Drug Biotransformation, Laboratory of Developmental Pharmacology, NICHD, NIH, Bethesda, Maryland 20892
- T. Rossman, Department of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, New York 10016
- J. Rydström, Department of Biochemistry, University of Stockholm, Arrhenius Laboratory, S-106 91 Stockholm, Sweden
- H. Shichi, Institute of Biological Sciences, Oakland University, Rochester, Michigan 48063
- C.-P. D. Tu, Department of Microbiology, Cell Biology, Biochemistry & Biophysics, Paul M. Althouse Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802
- J. von Borstel, Department of Genetics, University of Alberta, G216 Biological Sciences Centre, Edmonton T6G 2E9, Canada
- S. Waelsch, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, New York 10461
- M. R. Waterman, Department of Biochemistry, University of Texas, Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235
- W. W. Weber, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104
- H. Westphal, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, Maryland 20205
- J. E. Womack, Department of Veterinary Pathology, Texas A & M University, College Station, Texas 77843
- D. Wu, Department of Tumor Research, Fujian Medical College, Central 817 Road, Fuzhou, Fujian, China
- H. Yonekawa, Department of Biochemistry, Saitama Cancer Center Research Institute, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00137-12 LDP

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

GENETIC REGULATION OF DRUG-CONJUGATING ENZYMES

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. S. Owens	Head	LDP, NICHD
-----	-------------	------	------------

Others:	T. Kimura	Visiting Fellow	LDP, NICHD
	A. Karkowsky	Medical Staff Fellow	LDP, NICHD
	V. McCauley	Biological Aid	LDP, NICHD
	O. Michioka	Visiting Fellow	LDP, NICHD

## COOPERATING UNITS (if any)

D.W. Nebert &amp; coworkers, Section on Pharmacogenetics and Molecular Teratology,

LDP:NICHD:NIH

P.I. Mackenzie, Unit on Recombinant DNA &amp; the Conjugating Enzymes, LDP:NICHD:NIH

## LAB/BRANCH

Laboratory of Developmental Pharmacology

## SECTION

Section on Drug Biotransformation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.25

## PROFESSIONAL:

3.25

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The regulation of the family of UDP glucuronosyltransferase enzymes is being studied by means of DNA, RNA and protein chemistry. Different members of the transferase system are known to be induced by a number of different types of effector compounds; several such compounds used in these studies are phenobarbital, clofibrate, perfluorooctanoic acid, benzo[a]pyrene and 3-methylcholanthrene. A number of mouse transferase clones have been isolated and identified by hybridization to rat transferase clones and/or by immunoreactivity of protein expressed by a recombinant  $\lambda$ gt11 system. A clone  $\lambda$ gtUDPGT<sub>m</sub>-1 encoding the complete amino acid (530) sequence for mouse transferase is shown to contain an N-terminus membrane-insertion signal peptide, two potential glycosylation sites, and a carboxy-terminus membrane-spanning region. UDPGT<sub>m</sub>-1 encodes a 2200-nt mRNA and cross-hybridizes to a 2000-nt mRNA due to sequence homology in the 5' portion of the insert. The two mRNAs are induced 2.5- to 5-fold by the barbiturate phenobarbital, the hypolipidemic agents clofibrate and perfluorooctanoic acid, and the aromatic hydrocarbon benzo[a]pyrene. In a parallel manner, the same compounds induce bilirubin transferase activity. UDPGT<sub>m</sub>-1 hybridizes with a total of about 40 to 50 kb of genomic DNA. Upon screening a  $\lambda$ gt11 cDNA library constructed with 3-methylcholanthrene-induced mRNA, some 60 transferase-positive clones were identified by immunoreactivity. At least one clone (2400 bp) shows enhanced (5- to 10-fold) hybridization to 3-methylcholanthrene-induced mRNA and, thus, appears to be regulated by the aromatic hydrocarbon (Ah) locus. A human transferase clone UDPGT<sub>h</sub>-1 (2500-bp), identified by hybridization to UDPGT<sub>m</sub>-1, hybridizes to a 2500-bp mRNA and hybridizes with about 22 kb of genomic DNA. The substrate specificity of UDPGT<sub>m</sub>-1 is being investigated in a yeast expression vector.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00500-08 LDP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) RECEPTOR STRUCTURE AND FUNCTION		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	H. J. Eisen	Head LDP, NICHD
Others: See ATTACHMENT I		
COOPERATING UNITS (if any) D.W. Nebert & coworkers, Section on Pharmacogenetics & Molecular Teratology, LDP:NICHD:NIH S.S. Simons, Jr., Laboratory of Chemistry, NIADDK:NIH		
LAB/BRANCH Laboratory of Developmental Pharmacology		
SECTION Section on Regulation of Gene Expression		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 6.1	PROFESSIONAL 5.1	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)  Intracellular protein receptors have been identified for steroid hormones and polycyclic aromatic compounds. These compounds bind to their respective receptors; the ligand-receptor complexes interact with DNA and activate transcription of "target" genes such as cytochrome P450. The steroid hormone receptor gene family comprises homologous genes that code for at least three different steroid hormone receptors and the oncogene <u>erbA</u> . The <u>aromatic hydrocarbon</u> (Ah) receptor gene may also be a member of this gene family. We have previously demonstrated interesting genetic and biochemical differences between the Ah receptor and steroid hormone receptors. During the past year, we have completed 'mapping' of proteolytic products of the glucocorticoid receptor and have identified a unique cysteinyl residue at the C-terminus of the receptor which reacts covalently with the affinity labeling steroid [ <sup>3</sup> H]dexamethasone 21-mesylate. We have established within the LDP a <u>S.cerevisiae</u> cDNA expression system which we have used to express mouse and rat P450 cDNA, and which we will use also for expression of receptor cDNA clones. The availability of such an expression system should permit rapid screening of cDNA clones for TCDD-binding activity. We are currently pursuing the possibility that the Ah receptor shares homology with several domains that are conserved among estrogen, progesterone and glucocorticoid receptors from various species.		

## ATTACHMENT I - Others:

A. K. Bandyopadhyay	Expert	LDP, NICHD
T. Cresteil	Guest Researcher	LDP, NICHD
Anne G. Grant	Guest Researcher	LDP, NICHD
David A. W. Grant	Guest Researcher	LDP, NICHD
J.-Y. Lee	Visiting Fellow	LDP, NICHD
V. Rapic	Guest Researcher	LDP, NICHD
B. Raychaudhuri	Guest Researcher	LDP, NICHD
D. W. Towne	Chemist	LDP, NICHD



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00503-02 LDP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) REGULATION AND EXPRESSION OF THE UDP GLUCURONOSYLTRANSFERASE GENE FAMILY		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	P. I. Mackenzie	Visiting Associate LDP, NICHD
Others:	S. J. Haque	Visiting Fellow LDP, NICHD
COOPERATING UNITS (if any) D.W. Nebert & coworkers, Section on Pharmacogenetics & Molecular Teratology, LDP:NICHD:NIH I.S. Owens & coworkers, Section on Drug Biotransformation, LDP:NICHD:NIH		
LAB/BRANCH Laboratory of Developmental Pharmacology		
SECTION Unit on Recombinant DNA and the Conjugating Enzymes		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.8	PROFESSIONAL: 1.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The molecular mechanisms governing the regulation of the drug-detoxifying enzyme, UDP glucuronosyltransferase (transferase), and the structural differences between members of this family are being investigated in the rat. This animal, as exemplified by the Gunn rat, provides the only known animal model for investigating the defect in the glucuronidation of bilirubin and certain xenobiotics, characteristic of the Crigler-Najjar syndrome in humans. Certain strains of Wistar rat also have an inherited defect in the ability to glucuronidate the steroid hormone, androsterone. The isolation and sequencing of 7 cDNA clones has demonstrated the existence of at least 4 different forms of transferase belonging to two gene subfamilies. One clone pUDPGT<sub>r</sub>-2 encodes a phenobarbital-inducible form of transferase which glucuronidates 4-methylumbelliferone and the 17-OH position of testosterone and dihydrotestosterone. This was demonstrated by transfection into COS cells of the cDNA under the control of the SV40 promoter. This technique also demonstrated that a second clone, pUDPGT<sub>r</sub>-4, whose mRNA is not detected by phenobarbital or 3-methylcholanthrene, encodes a form of transferase which glucuronidates the 3-OH position of the androgens, androsterone and etiocholanolone. The substrate specificity of other transferases are currently under investigation. Sequence studies have also shown that the transferase forms encoded by the three cDNAs contain signal peptide and membrane anchoring regions, and potential asparagine-linked glycosylation sites. <u>In vitro</u> translation studies indicate that the signal sequence is cleaved during insertion into the endoplasmic reticulum and subsequent glycosylation occurs. Genomic clones to UDPGT<sub>r</sub>-4 have been isolated and are being sequenced. Genomic and cDNA clones will be utilized to study the regulation of each form as a function of age, tissue distribution and administration of prototypic inducers.           </p>		

# LABORATORY OF MOLECULAR GENETICS

- Z01 HD 00066-16 Control Mechanisms in Temperate Bacteriophage Lambda  
Robert A. Weisberg, Ph.D.
- Z01 HD 00067-18 Integration of Macromolecular Synthesis in E. coli  
Michael Cashel, M.D., Ph.D.
- Z01 HD 00068-15 Factors Influencing Genetic Transcription-Initiation  
and Termination  
Robert J. Crouch, Ph.D.
- Z01 HD 00069-14 Molecular Aspects of the Replication of Enveloped  
Animal RNA Viruses  
Judith G. Levin, Ph.D.
- Z01 HD 00071-14 Oncogenesis and Insertion Mediated Mutagenesis  
in Transgenic Mice  
Heiner Westphal, M.D.
- Z01-HD 01001-04 Gene Organization and Expression in Drosophila  
Igor B. Dawid, Ph.D.
- Z01 HD 01002-04 Gene Expression During Embryonic Development of  
Xenopus Laevis  
Igor B. Dawid, Ph.D.
- Z01 HD 01003-04 Genetic Control of Oncogenesis  
Hiroto Okayama, M.D., Ph.D.
- Z01 HD 01004-03 Regulation of Amino Acid Biosynthetic Genes in  
Saccharomyces Cerevisiae  
Alan G. Hinnebusch, Ph.D.





NICHD ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Laboratory of Molecular Genetics

During the past year, the members of the Laboratory have continued in their research programs that utilize the methods of molecular biology to gain insights into mechanisms of regulation of the genetic material in cell function and heredity, especially in the responses to nutritional state and during the multifaceted events of differentiation. As in past years, different biological systems were studied, each with its unique characteristics, special points of interest, and particular suitability for answering specific questions. Advances have been made in many areas. Particularly interesting are results obtained in the study of transgenic mice which highlight the correlation between the targeted expression of an oncogene with tumorigenesis and the relationship between transformation and differentiation; and the detailed study of the mechanism of translational regulation of expression of a transcriptional regulator in yeast, giving the first detailed insight into a novel and probably general type of regulatory network in a eukaryotic cell.

Description of the research program in the Laboratory of Molecular Genetics

Gene regulation in Transgenic Mice

Heiner Westphal and his colleagues have established in the Laboratory the methodology of inserting genetic material into the mouse germline to produce transgenic mice. In general, this methodology may yield results in three different areas, all of which are represented in the work of this group: (i) The determination of the control regions of individual genes that are required for temporal and tissue-specific regulation in the whole animal; (ii) insertion of genes that have a dominant effect on the transgenic animal; and (iii) production of mutants through insertional mutagenesis. Results relevant to point (i) have been reported in the previous Annual Report; the clearest result in this area and the one relevant to current work is the observation, made in collaboration with Joram Piatigorsky and his colleagues in the National Eye Institute, that a region of only 409 nucleotides from the 5' region of the  $\alpha$ A-crystallin gene can direct the proper expression of a marker gene (chloramphenicol acetyltransferase, CAT) in the transgenic mouse. CAT activity was found only in the lens fiber and epithelial cells beginning at 12.5 days of gestation, showing precise temporal and spatial regulation of the  $\alpha$ A-crystallin/CAT fusion gene.

The results summarized above define a highly specific promoter element whose properties were used to target the expression of an oncogene to a certain tissue with the aim to produce a dominant phenotype in a transgenic strain. The SV40 T antigen gene was fused to the  $\alpha$ A-crystallin promoter and inserted into the germ line. Such animals reproducibly exhibited cataracts in both eyes as soon as newborns open their eyes. Histological studies employing standard staining, as well as immunocytochemistry and in situ hybridization with nucleic acid probes, showed that the lens of these animals was filled with transformed cells. Such cells stained for both T antigen and crystallins, but crystallin staining was patchy. Transformed cells were first visible at the time of

initial crystallin expression; thus, it appears that lens cells begin to differentiate, thus turning on crystallin genes and with them the hybrid T-antigen gene. Very shortly after this time, phenotypic effects of transformation become detectable. The affected mice usually die at an age of 3 to 4 months. Removal of the eyes at birth appears to prolong their lifespan; one such operated animal died at 6 months with evidence of multiple tumors, all of which expressed T antigen. These results are of considerable interest from several points of view, among them the generation of lens tumors which do not occur under natural conditions in any animal including humans. Further, the time course of transformation in a differentiating tissue, and the apparent suppression of the differentiated phenotype by transformation (reduction in crystallin expression) can be studied both in vivo and in vitro in cells cultured from the affected lens.

Observations relevant to the third point mentioned at the outset have also been made. Two dominant and one recessive trait have been observed among the transgenic mice studied so far. The dominant trait in both cases is small litter size which has been explained by cytogenetic analysis as being due to chromosomal translocations. One translocation which has been studied in detail is linked to the inserted foreign DNA and is expected to have been precipitated by the insertion event. The recessive trait is syndactyly; again, it may be hypothesized that the mutation has been generated by the insertion of the foreign DNA into a locus involved with limb formation. The great advantage of mutations generated through DNA insertion as opposed to more traditional mutagens is that the introduced DNA and regions flanking it can be isolated from a library of the mutant genome by virtue of the available transgenic sequence. Such approaches might allow the study of developmentally interesting genes with no known products in a mammalian species.

In sum, the results obtained demonstrate that the transgenic mouse system provides an entry into the study of gene regulation and developmental mechanisms in the mouse that goes well beyond anything possible before introduction of this methodology.

#### The Mechanism of General Amino Acid Control in Yeast

Alan Hinnebusch and his colleagues have continued their studies on the system in yeast that regulates production of more than 30 amino acid biosynthetic enzymes according to the availability of amino acids in the medium. It has been shown previously that the GCN4 locus is the proximal regulatory element of the many target genes; there is strong evidence that the GCN4 product is a transcriptional regulator of these genes. It had also been shown that GCN4 expression is regulated at the translational level and that several other loci, to be discussed further below, are involved in this regulation. The mechanism of GCN4 regulation was explored further in the current year. Four short open reading frames (ORF's) occur in the long leader sequence of the GCN4 mRNA; their total deletion leads to unregulated high expression of the GCN4 product. Point mutagenesis affecting each of the ORF AUG's individually and in groups was carried out and demonstrated that either of the two 3'-proximal ORF's is necessary and sufficient for repression of GCN4. The two 5'-proximal ORF's do not have such a repressing role but rather appear to act positively: They are required for efficient GCN4 expression during starvation. The interaction between the 3' and 5' ORF's is mediated by another locus, GCD1, that acts further upstream in the hierarchy of general control. GCD1 is required for



normal repression of GCN4 when amino acids are available; in gcd mutants GCN4 and consequently general control genes, are constitutively derepressed. This function appears to be mediated through the 5'-proximal ORF's since GCN4 constructs which have only one 3'-proximal ORF are always repressed, even in gcd1 mutants.

The path from external signal - presence or absence of amino acids - to regulation of general control genes is mediated by several loci. GCD1, the locus mentioned above, is just one of a class of genes required for repression of GCN4. A genetic study carried out by S. Harashima and A. Hinnebusch identified four new GCD loci in addition to isolating new alleles in the previously known locus. All of these five genes are required to repress translation of GCN4 under conditions of amino acid availability. Mutations in these five genes have pleiotropic effects including increased sensitivity to aminoglycosidic antibiotics, suggesting that the wild type gene products are involved in protein synthesis; this makes sense for components that mediate translational regulation. The GCD1 and GCD12 functions are required for progression through the cell cycle.

Mutations in GCD genes overcome the effects of mutations in the positive regulators GCN2 and GCN3, suggesting that these GCN genes act by antagonism of GCD-mediated negative regulation of GCN4. E. Hannig and S. Harashima have found that expression of both the regulatory and temperature sensitive cell-cycle phenotypes of certain gcd1 and gcd12 mutations requires a null allele of gcn3: In a GCN3<sup>+</sup> strain, the phenotypes of these gcd mutations are suppressed. Importantly, gcn3-102, which is completely defective for positive regulation of GCN4, behaves more like GCN3<sup>+</sup> and suppresses these gcd mutations. The allele specificity of these interactions suggests that the GCN3<sup>+</sup> or gcn3-102 proteins can interact with and stabilize thermolabile gcd proteins. In this model, a complex between GCN3 and GCD proteins is responsible for both coordinating the translational efficiency of GCN4 mRNA with amino acid availability and for an essential function required for progression through the cell cycle.

The particular interest of this work stems from its detailed analysis of the mechanism of translational control of transcription in a eukaryotic cell. In prokaryotes, modulation of translation of mRNA while it is still being synthesized on its DNA template, provides for a regulatory mechanism that has been understood for some time. The nuclear/cytoplasmic division in eukaryotes make this type of mechanism impossible; the work summarized here provides insights into a mechanism in which translational regulation of a transcriptional activator links the events of protein and RNA synthesis in a regulatory loop.

#### Gene Expression during Embryonic Development of *Xenopus laevis*

Igor Dawid, Tom Sargent and their colleagues have continued their studies on molecular mechanisms of development in *Xenopus*. A set of genes that are expressed for the first time in late blastula to gastrula stages has been used to generate markers for the earliest cell type-specific functions in the embryo. A major focus of recent work has been the family of embryo- and tadpole-specific keratin genes. Keratins are the protein subunits of intermediate filaments in epithelial cells, constituting major components of the cytoskeleton. Structural studies have revealed the remarkable complexity of this gene family in the frog. Seven distinct keratin genes have been isolated in this laboratory and shown to be arranged in three subfamilies. The expression of these



genes is limited to embryos and tadpoles while other keratin genes, five of which have been isolated by a different laboratory, are active in adult skin and other epithelia. These studies have been complemented by 2-dimensional gel electrophoretic analysis of proteins, demonstrating the complexity of the keratin family by another technique. The correspondence between some of the 2-D spots and certain cloned cDNAs has been demonstrated by hybrid arrest translation. The 2-D gel analysis suggests that the major, but not all of the embryonic keratin genes have been isolated.

The most interesting aspect of the study of keratin gene expression in the embryo concerns the use of these genes in the analysis of ectoderm differentiation. Previously, this laboratory has demonstrated that activation of keratin genes does not require cell-cell interactions but is an autonomous function of embryonic cells. In the current year, detailed studies on the localization of accumulation of keratin mRNA and protein in the embryo were carried out by two complementary techniques, in situ hybridization in sections and immunofluorescence. The former technique had to be modified to allow its effective use in early frog material; advances in this direction have opened up important avenues for the study of amphibian embryogenesis. The experiments show that keratin genes are activated in the outer layer of the ectoderm in late blastula. Keratin filaments begin to form primarily at the outer edge of the ectodermal cells shortly after gene activation; a much looser net of filaments forms in the interior of the outer cells and throughout the inner cell layer of the ectoderm. During gastrulation, the involuting chordamesoderm induces neural development in a specific region of the ectoderm. Prior to contact with the mesoderm, the prospective neural ectoderm does initiate keratin gene expression; however, shortly after contact with the mesoderm, the accumulation of keratin mRNA and protein ceases and the previously formed material decays. These results led to the conclusion that keratin gene activation in the embryo is initiated in the ectoderm as a result of preexisting localized information, but subsequent activity is modulated by inductive interactions. Cessation of keratin mRNA accumulation is the earliest available molecular marker for the differentiation of the central nervous system in the amphibian. The use of this marker yielded the conclusion that pre-neural and pre-epidermal ectoderm express some of the same genes which distinguish the ectoderm from endoderm and presumptive mesoderm. Subsequently, probably as a result of inductive influences generated from the chordamesoderm, the neural and epidermal pathways diverge as exemplified in increasingly different sets of tissue-specific products. It is hoped that the isolation of embryonic neural specific genes will allow the further analysis of these phenomena in the near future.

#### Molecular Genetics of fs(1)h, a Maternal Effect Homeotic Gene in Drosophila

Homeotic genes have attracted much attention because they appear to encode factors that are involved in the specification of segment identity in the fly. There is much evidence to support the notion that homeotic genes control entire pathways of tissue differentiation, and thus constitute master control loci in development. The homeotic loci studied in many laboratories have exclusively or predominantly zygotic functions, i.e., the genotype of an individual determines its phenotype. A large amount of developmental work over many years has suggested that there are factors stored in the oocyte that control subsequent development; genes encoding such factors would constitute maternal effect loci with profound effects on development. The locus studied by Igor Dawid and his colleagues, fs(1)h, has such effects: It encodes a product or products which

is stored in the egg and is required for embryogenesis. Under certain conditions, mutant females are sterile, i.e., they lay eggs that die as early embryos, but under different conditions they produce progeny that exhibit transformation of body parts similar to those seen in bithorax mutations. These transformation are greatly enhanced in flies that also carry mutations or hypodosage of other homeotic loci, especially of the trithorax (trx) gene. A molecular study of fs(1)h is therefore a promising avenue to understanding certain aspects of developmental regulation through oocyte factors.

The genome region containing fs(1)h has been cloned previously by chromosomal walking. Recently, transcription analysis demonstrated considerable complexity in the RNA products of this region. Two major ovarian RNAs, five larval RNAs and one pupal RNA, have been identified; they are generated from the same 20 kb-long region by alternate splicing, alternate 3' ends, and possibly alternate starting points. cDNA clones have been isolated and are being sequenced at present with the aim to predict protein products for the locus. Fusion proteins between portions of fs(1)h and  $\beta$ -galactosidase have been generated and are being expressed in *E. coli*; the fusion proteins will be injected into rabbits for production of antibodies that should aid in the analysis of the natural products of the fs(1)h gene.

#### Integration of Macromolecular Synthesis in *E. coli*

Michael Cashel and his colleagues have continued their studies on global control of metabolism in *E. coli*. This term implies a regulatory system which allows the cell to respond to external conditions, e.g., nutritional deprivation, with adjustments in overall gene expression and metabolism. Different types of mechanisms are known that mediate the control of many genes in such responses; a major mechanism and the one studied by Cashel's group involves the unusual nucleotide ppGpp. High levels of ppGpp in the cell are correlated with inhibition of transcription of rRNA, tRNA and other genes encoding components of the protein synthesis machinery, but with stimulation of expression of amino acid biosynthetic enzymes and heat shock proteins, and enhanced proteolysis and accuracy of translation. The phenomena studied in detail in this laboratory concern inhibition of rRNA synthesis and stimulation of histidine operon expression.

An important tool in this work has been the development of bacterial strains in which the level of ppGpp can be varied experimentally without changing the richness of the growth media. This has become possible through the study of the spoT gene which encodes a 3'-pyrophosphatase that accounts for the major pathway of degradation of ppGpp. The spoT gene has been studied in its own right, yielding its sequence and allowing the construction of deletions and insertions that will be used in studying the functions of this gene further. The use of spoT mutations in changing in vivo ppGpp concentrations at will resulted in the isolation of rRNA promoter mutations that are insensitive to ppGpp; these mutations will be most helpful in future work. Further, it could be shown that the activity of the rRNA gene promoter P1 correlates inversely with ppGpp concentrations over the entire physiological range, suggesting that ppGpp interacts directly with the promoter.

Detailed studies have continued on several unusual features of rRNA operons, including dual promoters, antitermination, and dual terminators that apparently can stop antiterminated transcripts (supertermination). Novel results in this



area includes the finding of an antitermination region just upstream of the 23S RNA gene, in an analogous position to the previously detected region upstream of the 16S RNA gene. The problem why two antitermination regions occur is being studied.

#### Enzymes Involved in RNA Processing

**Robert Crouch** directs a program with the aim to analyze certain aspects of RNA processing. It has been clear for some time that all RNA molecules are modified in various ways after they are synthesized and before they function: the polymerization of nucleotides into an RNA chain is but the initial step in its production, and subsequent events are crucial in generating a functional entity. Certain RNAs act as primers in replication, and enzymatic mechanisms must exist that remove these primers after they have completed their function. Crouch and his colleagues have focused on an enzyme, RNase H, that appears to be involved in this event. RNase H, an enzyme that digests the RNA strand of RNA/DNA hybrids occurs in all cells from bacteria to mammals, but its function is not fully understood.

The RNase H gene has been cloned from *E. coli*, *Salmonella*, and yeast, and the sequences of these genes have been determined. Comparisons of these species and mutant forms of the *E. coli* enzyme have provided insights into enzyme structure. In yeast, three distinct RNase H proteins have been found. This fact may explain the finding that inactivation of the RNase H gene that has been cloned does not lead to any obvious phenotype.

The second area of interest in this research group concerns research on RNA processing in mammalian cells. A mutant line of BHK cells impaired in the formation of 28S rRNA due to a processing defect has been studied. Transfection of cDNA generated from normal mouse cells has resulted in the correction of the processing defect, suggesting that the wild type gene has been introduced into the mutant cell. This result should help in determining the nature of the function which is defective in the mutant line and through it aid in analyzing the mechanism of rRNA processing.

#### Control Mechanisms in Bacteriophage Lambda

**Robert Weisberg** and his colleagues have continued their studies on mechanisms of recombination and gene expression of bacteriophage  $\lambda$ . An important component of regulation of gene expression is termination of transcription at specific sites in the DNA called terminators. Termination may be enhanced or suppressed by protein factors; work in the current year has provided evidence for the view that the mechanism of termination enhancement and suppression are closely related. This conclusion stems from work with a protein encoded by the  $\lambda$ -related phage HK022, called Nun, which promotes termination or antitermination, depending on the gene affected. Nun appears to act as an antitermination factor on its own genome, HK022; on the related phage  $\lambda$  the same protein promotes termination. In the latter action, it requires the same host cell factors and sites that are required for antitermination promoted by the  $\lambda$  N protein. These findings suggest a fundamental unity in the mechanisms of termination and antitermination.

A major interest of this research group has been the mechanism of recombination, specifically during integration and excision of phage  $\lambda$  from the bacte-



rial chromosome. Site-specific recombination promoted by the int protein occurs at attachment sites that contain a central core of 7 homologous nucleotides. Mismatched attachment sites do not recombine efficiently. Recent work suggests that even mismatched sites form the recombinational intermediate called a Holiday structure, but that they are resolved only to parental rather than recombinant molecules. In the interaction between homologous sites branch migration across the region of homology allows resolution towards the recombinant at high frequency; facilitation of branch migration may be the key function of the homologous core region of attachment sites.

#### Molecular Aspects of Replication of Enveloped Animal RNA Viruses

**Judith Levin's** group is concerned with the mechanism of replication of enveloped RNA viruses, specifically murine leukemia viruses (MuLV). Studies on the RNA tumor viruses are of particular interest since these viruses establish a chronic infection in the host cell by integrating viral genetic information into the host chromosome, often disrupting normal gene function. A detailed understanding of the steps involved in viral replication may permit the design of agents which would limit the oncogenic potential of these viruses. Moreover, evidence is accumulating that a number of eukaryotic genetic elements have been created by transfer of information from RNA to DNA during the development of the eukaryotic genome.

Current interest in this program is focused on the process of reverse transcription in an effort to correlate genetic structure with enzymatic function. Portions of the pol gene have been expressed in *E. coli* and the products were used to generate antibodies specific for polymerase and endonuclease. These reagents have proved valuable in dissecting the relationships between these gene products. Recently, studies with an antiserum directed against endonuclease alone have led to the observation that the viral reverse transcriptase and endonuclease can be associated as a complex involving both non-covalent and disulfide bonds. The exact nature of this association within the virion is under investigation. The sera are also being used to define the defect in a viral mutant with an in-frame deletion in the endonuclease coding region. In addition, a clone expressing only reverse transcriptase sequences has been used to make a monospecific anti-polymerase serum and to provide a source of highly active enzyme. Large scale preparations of bacterial extracts of this clone have now been extensively purified by standard biochemical procedures and characterization for the purified bacterially expressed reverse transcriptase is in progress.

#### Genetic Control of Oncogenesis

**Hiroto Okayama** and his colleagues pursue the question of oncogenesis through the approach to clone genes that are required for transformation. To this end they have developed methods for the insertion of cDNA clones in an expressible form into cultured cells.

This program has transferred to the NIMH during the current reporting period and is terminated in this Laboratory.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00066-16 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control Mechanisms in Temperate Bacteriophage Lambda

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert A. Weisberg Head LMG, NICHD

Others: Bernard de Massey Visiting Fellow LMG, NICHD  
Jacques Oberto Visiting Fellow LMG, NICHD  
Ezra Yagil Visiting Scientist LMG, NICHD  
Sieghild Sloan Microbiologist LMG, NICHD

## COOPERATING UNITS (if any)

Institute of Cancer Research; Columbia University, NY, NY (Dr. Max Gottesman)  
Laboratory of Molecular Biology; NIMH (Dr. Howard Nash)  
Molecular Genetics Inc., Minnetonka, MN (Dr. W. Delorbe)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Microbial Genetics

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

4.3

## PROFESSIONAL:

3.3

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have discovered and begun the characterization of a protein called Nun, which appears able to promote both termination and antitermination of transcription. Nun, encoded by the  $\lambda$ -related bacteriophage HK022, promotes transcription termination on a  $\lambda$  template, but appears to suppress termination on an HK022 template. Suppression of termination by Nun appears to be analogous to the action of several previously characterized bacteriophage antitermination factors such as the phage  $\lambda$  N protein: the location of the nun gene in HK022, between a major early promoter (pL) and a transcription terminator, corresponds to that of the N gene in  $\lambda$ . We have also shown that transcription termination by Nun on a  $\lambda$  template requires the same protein (Nus factors) and sites (nut sites) that are required for N-promoted antitermination on the same template. These findings suggest a fundamental unity in the mechanisms of termination and antitermination.

The frequency of int-promoted recombination between two phage  $\lambda$  attachment sites is reduced when the nucleotide sequence of one of the sites differs from that of the other within a 7 bp segment called the overlap region. Recombination is thought to proceed through a branched DNA intermediate called a Holliday structure that is resolved to recombinant products. We have shown that synthetic Holliday structures that contain one copy of an overlap region mutation called safG and one copy of a wild type overlap region are not converted to recombinant molecules by int protein, but instead are converted back to parental molecules. Work in another laboratory has shown that safG nonhomology does not prevent the formation of Holliday structures by int protein. Together, these findings argue against a model in which direct interaction between homologous nucleotides of the overlap regions is required for synapsis. We now favor a model in which such homologous interaction is required for resolution of the recombination intermediate to recombinant products. A simple molecular mechanism for such an interaction is branch migration of the Holliday structure from one end of the overlap region to the other.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00067-18 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Integration of Macromolecular Synthesis in *E. coli*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Michael Cashel Head LMG, NICHD

Others: Kenneth E. Rudd Staff Fellow LMG, NICHD  
 Edoardo Sarubbi Visiting Fellow LMG, NICHD  
 Shaw Chen Clinical Associate LMG, NICHD  
 Chikh Bengra Visiting Fellow LMG, NICHD  
 Eric Devaney Chemist LMG, NICHD

## COOPERATING UNITS (if any)

Dept. Cellular Biochemistry, Hadassah Medical School (Dr. Gad Glaser);  
 Dept. Microbiology, University of Connecticut Health Center, Farmington,  
 Conn. (Dr. Asis Das).

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Molecular Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

5.7

## PROFESSIONAL:

4.9

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

We wish to understand how the expression of the *Escherichia coli* genome is coordinately regulated during growth rate transitions accompanying physiological and nutritional stress. Interest centers on the pleiotropic regulatory effects mediated by guanosine 3',5'-bispyrophosphate (ppGpp) and focuses on a primary regulatory target inhibited by ppGpp, ribosomal RNA operon transcription. Normally, ppGpp accumulates to high levels during the stringent response to amino acid starvation and to varying levels that are correlated inversely with nutritional abundance and growth rate. We have devised a means of varying steady state levels of ppGpp independent of nutritional adequacy by isolating mutants in the spoT gene with varying degrees of defects. The spoT gene encodes a ppGpp 3'-pyrophosphatase. Studies with these isogenic strains show that a strong inverse correlation between rRNA P1 promoter activity and ppGpp levels holds over the full physiological range of ppGpp concentration, including those characteristic of normal growth. A similar correlation applies for growth rate itself. The simplicity of this relationship suggests a direct interaction of ppGpp as a negative effector with the P1 promoter during normal growth as well as during the stringent response. This possibility is being tested by the derivation of ppGpp insensitive P1 promoter mutants that have been fused to a galactokinase indicator gene. The spoT gene has been localized within a new operon and is being characterized by chromosomal insertion-deletion analysis. Ribosomal RNA operons display several unusual transcriptional features, including antitermination, dual promoters with supercoil-dependent activities, and presumably the ability to stop an antiterminating transcript. We are continuing to dissect out these features and have found a new region of antitermination just upstream of the 23S gene in a position analogous to the known region just upstream of the 16S gene. We have also found antitermination sequences in plasmids are unstable. Wild-type antitermination sequences are considerably more active than spontaneous stable variants.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00068-15 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Influencing Genetic Transcription-Initiation and Termination

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.J. Crouch Research Chemist LMG, NICHD

Others: M. Itaya Visiting Fellow LMG, NICHD  
L. Lempereur Visiting Fellow LMG, NICHD  
B. Stevens-Klapholtz Visiting Associate LMG, NICHD  
A. Hinnebusch Senior Staff Fellow LMG, NICHD

## COOPERATING UNITS (if any)

P. Heiter Johns Hopkins University  
S.K. Dutta Howard University

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Molecular Regulation

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.25

## PROFESSIONAL:

2.25

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RNA plays an important role in cellular regulation -- either by its presence in active form or by its total absence. It has been known for several years that transcription of DNA does not necessarily lead to productive, mature RNA molecules. Cleavage of these RNA molecules often is required for the RNA molecules to mature or to act as an intermediate in other processes (e.g., priming of DNA replication). These cleavage events are a subset of a general maturation pathway known as RNA processing. Work of this Intramural Research Project is concerned with two types of RNA processing, generation of RNA primers for DNA replication and ribosomal RNA processing in higher eukaryotes. We have studied the relationship between amino acid sequence and enzymatic activity of ribonuclease H (RNase H) by examination of mutants of Escherichia coli with altered RNase H activities, by determination of the amino acid sequence of Salmonella typhimurium RNase H and a yeast RNase H. The gene coding for the yeast RNase H has been located on the yeast genetic map to the right arm of chromosome XIII about 250 kilobase from the telomere. Inactivation of the yeast RNase H gene produces strains that exhibit normal growth phenotypes. The processing of ribosomal RNA in a mutant BHK cell line has also been studied. The temperature sensitive phenotype has been corrected by transfection of the mutant cells with a cDNA library from mouse.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00069-14 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Aspects of the Replication of Enveloped Animal RNA Viruses

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Judith G. Levin	Research Biochemist	LMG, NICHD
Others:	Stella C. Hu	Chemist	LMG, NICHD
	Michael Seddon	Bio Aid	LMG, NICHD
	Steve Joe	SIS	LMG, NICHD

## COOPERATING UNITS (if any)

NCI - FCRF (Don Court); Basic Research Program, LBI, NCI-FCRF  
(Alan Rein); PRI-FCRF (Martin Zweig)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Unit on Viral Gene Regulation (Developmental Biology Section)

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

1.7

## PROFESSIONAL

1.0

## OTHER

0.7

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

The goal of this project is to define the molecular mechanisms involved in the replication of enveloped RNA viruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the MuLV pol gene and on correlation of genetic structure with pol-associated enzymatic functions. Molecular clones containing MuLV reverse transcriptase and/or endonuclease gene segments have been expressed in E. coli and specific antisera have been generated against the expressed bacterial proteins. These antisera are useful reagents for characterizing viral pol proteins, and serve as probes for the protein structure of the virus. Recently, studies with an antiserum directed against endonuclease alone have led to the observation that the viral reverse transcriptase and endonuclease can be associated as a complex involving both non-covalent and disulfide bonds. The exact nature of this association within the virion is under investigation. The sera are also being used to define the defect in a viral mutant with an in-frame deletion in the endonuclease coding region. In addition, a clone expressing only reverse transcriptase sequences has been used to make a monospecific anti-polymerase serum and to provide a source of highly active enzyme. Large scale preparations of bacterial extracts of this clone have now been extensively purified by standard biochemical procedures and characterization of the purified bacterially expressed reverse transcriptase is in progress.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00071-14 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oncogenesis and Insertion Mediated Mutagenesis in Transgenic Mice

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. Westphal, Head

Others: J. Khillan, Visiting Associate

B. Krippl, Visiting Associate

K. Mahon, Staff Fellow

A. Griep, Staff Fellow

M. Mangano, Guest Researcher

S.P. Lai, Chemist

E. Lee, Veterinarian

A. Miller, Biologist

A. Dey, Visiting Fellow

T. Nakamura, Visiting Fellow

A. Graessmann, Guest Researcher

(All listed personnel affiliated with LMG/NICHD)

## COOPERATING UNITS (if any)

NEI, NIH (J. Piatigorsky)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Animal Viruses

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

8.5

## PROFESSIONAL:

6.2

## OTHER:

2.3

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Our laboratory investigates mechanisms of gene control in the living mammalian organism. Specific genes are introduced by microinjection into the germ line of mice, and expression of these genes is assayed in the resulting transgenic animals. To this end, organ screens are performed at various stages of development from fetus to adult. Two problems were studied. The first dealt with oncogenesis in the transgenic animal. A fused gene, consisting of the mouse  $\alpha$  crystallin promoter and the coding sequences of the SV40 tumor antigens, was inserted in the mouse germ line. Lens tumors developed in all mouse lines generated so far. Tumor cells expressed the SV40 T antigen. The onset of cell transformation coincided with lens differentiation during mid-gestation. The second problem concerns insertional mutagenesis observed in one of our strains of transgenic mice. A dominant phenotype of small litter size was shown to coincide with a chromosome 6/17 reciprocal translocation, and the gene insert was localized by in situ hybridization to one of the rearranged chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 01001-04 LMG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Gene Organization and Expression in Drosophila		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	I. Dawid	Head LMG, NICHD
Others:	M. Rebbert	Chemist LMG, NICHD
	B. Mozer	Biologist LMG, NICHD
	F. Forquignon	Visiting Associate LMG, NICHD
	S. Haynes	Senior Staff Fellow LMG, NICHD
	N. Bhatia-Dey	Guest Researcher LMG, NICHD
	D.-H. Huang	Guest Researcher LMG, NICHD
	D. Davis	SIS LMG, NICHD
COOPERATING UNITS (if any) Centre Genetique Moleculaire, CNRS, G-fi-sur-Yvette, France (M. Gans and F. Forquignon)		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Section on Developmental Biology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL.	OTHER:
4.0	1.9	2.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The maternal effect homeotic gene <u>fs(1)h</u> of <u>Drosophila</u> has been studied by genetic and molecular techniques. The gene has been cloned previously. In the current year, the zygotic effect of <u>fs(1)h</u> mutations has been analyzed, indicating the <u>fs(1)<sup>+</sup></u> gene product is required through larval stages. Analysis of <u>fs(1)h</u> transcripts revealed a complex pattern: In the ovary major 7.6 and 5.9 kb RNAs occur, in larvae 5 different molecules are found including two species between 8 and 9 kb, and a single 2.4 kb transcript occurs in pupae. Cloned cDNAs corresponding to most sequences of the ovarian RNAs have been isolated. Analysis of these clones indicates the presence of at least 5 introns in the <u>fs(1)h</u> gene, and suggests that the 7.6 and 5.9 kb RNAs have a common 5' region but different 3' segments. These results, together with earlier genetic data, suggest that the proximal (5') region of the locus is involved in segment identity (i.e., responsible for homeotic effects), while the distal (3') region carries functions for embryo survival. The <u>fs(1)h</u> transcripts are uniformly distributed in the oocyte and embryo, as determined by <u>in situ</u> hybridization.           </p> <p>             A <u>repetitive</u> sequence, named <u>pen</u>, has been characterized in the <u>D. melanogaster</u> genome. Unlike most repeated elements in this animal, the <u>pen</u> sequence is not precisely defined in its length or sequence, but rather constitutes a sequence motif composed of <u>clustered GGX</u> triplets, where X can be any nucleotide. This sequence could encode poly-glycine stretches that might form <u>flexible protein domains</u>.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01002-04 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Expression During Embryonic Development of *Xenopus Laevis*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: I.B. Dawid, Head (All personnel listed below are associated with LMG/NICHD)

Others: T. Sargent, Senior Staff Fellow  
S. Miyatani, Visiting Fellow  
J. Winkles, Staff Fellow  
M. Jamrich, Visiting Associate  
E. Jonas, Visiting Fellow  
A. Cheng, Biologist

G. Michaels, Staff Fellow  
A. Krasner, Guest Researcher  
S. LaFlamme, Guest Researcher  
D. Davis, SIS  
M. Rebbert, Chemist

## COOPERATING UNITS (if any)

ERRB, NICHD, NIH (H.-C. Chen and J.L. Morell)  
LNN, NICHD (L. Charnas and H. Gainer)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Developmental Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

8.2

## PROFESSIONAL:

6.4

## OTHER:

1.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This program aims to elucidate molecular events in early amphibian embryogenesis; a family of genes encoding embryo and larval specific keratins have been the primary focus of recent attention. Analysis of these genes has revealed the presence of three subfamilies, named XK70, XK81 (both acidic, type I keratins), and XK76 (a more basic, type II keratin). Genomic and/or cDNA clones have been isolated for 4 members of the XK81 subfamily and 2 members of the XK70 subfamily. Genomic sequences obtained for 3 XK81 genes reveal conserved 5' flanking regions that could have regulatory functions. Genomic clones corresponding to XK70 have also been isolated and sequenced. Constructs containing the 5' flanking region from the XK81A1 gene attached to the CAT marker gene have been injected into frog embryos, resulting in expression during embryogenesis of CAT enzyme activity.

The regions of accumulation of keratins and keratin mRNAs in the frog embryo have been studied by in situ hybridization and immunofluorescence. Keratin mRNA and keratins accumulate primarily in the outer layer of the ectoderm at early gastrula and the epidermis during subsequent differentiation. During gastrulation, neural induction of an ectodermal region by chrodamesoderm leads to formation of the central nervous system; this inductive event is accompanied by immediate cessation of keratin mRNA accumulation in this region. Regulation of keratin synthesis is the earliest available molecular marker for neural induction and provides an approach to the study of neurogenesis.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01003-04 LMG
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Control of Oncogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Hiroto Okayama	Visiting Scientist LMG, NICHD
Others:	Masashi Kawaichi	Visiting Associate LMG, NICHD
	Claudia Chen	Biologist LMG, NICHD
	Noriko Nukiwa	Guest Researcher LMG, NICHD
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Section on Developmental Biology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS. 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Oncogenes seem to transform cells through a common pathway(s), which perhaps involves cellular growth regulatory systems including growth control coupled to cell-cell contacts, growth factor production and cell cycle controls. In order to elucidate this pathway, we have been attempting to molecularly clone cellular genes having transforming potential by utilizing the cDNA expression cloning system we developed.</u> </p> <p> <u>Transfection of NIH3T3 cells with a SV40-transformed human fibroblast cDNA library constructed in a mammalian expression vector have yielded 30 independent morphologically transformed colonies. In order to clone integrated transforming cDNAs, lambda genomic libraries were constructed with total genomic DNAs of each transformant and are currently being screened by plaque hybridization with the vector sequence as a probe. Overlapping lambda clones have been so far recovered from two primary transformants, and found to be able to transform cells. The lambda clones can induce foci but little if any morphological changes. These clones do not have any significant homology to major known oncogenes. Characterization of these clones is currently in progress. The clone reported last year was found to contain a part of the SV40 VP3 capsid protein gene. Preliminary experiments indicate that the intact VP3 gene may have weak transforming activity.</u> </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01004-03 LMG

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Amino Acid Biosynthetic Gene in *Saccharomyces Cerevisiae*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Alan G. Hinnebusch	Senior Staff Fellow	LMG, NICHD
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Others:	Ernest Hannig	Staff Fellow	LMG, NICHD
	Satoshi Harashima	Visiting Associate	LMG, NICHD
	Alice Ma	Biologist	LMG, NICHD
	Peter Muller	Visiting Fellow	LMG, NICHD
	Gary Fabian	Staff Fellow	LMG, NICHD
	Chris Paddon	Visiting Associate	LMG, NICHD

## COOPERATING UNITS (if any)

NONE

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Section on Developmental Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6.1

## PROFESSIONAL:

5.1

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying a regulatory system in *Saccharomyces cerevisiae*, known as general amino acid control, which couples the rate of transcription of a large number of unlinked amino acid biosynthetic genes to amino acid availability. We have shown previously that expression of GCN4, the direct positive effector in this system, is controlled by amino acid availability. This regulation of GCN4 operates at the translational level and is mediated by cis-acting sequences in the 5' end of GCN4 mRNA. GCN4 expression is also controlled by several trans-acting factors, some of which promote GCN4 expression in starvation conditions (GCN) and others which are required for repression in non-starvation conditions (GCD). In the past year, we have completed our analysis of a large number of point mutations which remove the AUG initiation codons of four small open-reading frames located in the 5' leader of GCN4 mRNA. Our results show that these open-reading-frames are critical for the translational control of GCN4, that the individual open-reading-frames have distinct functions, and that interactions between the open-reading-frames are essential for a proper regulatory response. Furthermore, it appears that the GCN and GCD factors regulate GCN4 by controlling the interactions between the upstream open-reading-frames. We have isolated many new gcd regulatory mutations in five unlinked genes and find that all of these new mutations impair the translational regulation of GCN4. Interestingly, many of the gcd mutations are conditional lethals. In particular, mutations in GCD1 and GCD12 lead to arrest in the G1 period of the cell cycle at 36°. The gcd1 and gcd12 mutations also show an unexpected allele-specific interaction with previously isolated mutations in the positive regulatory gene GCN3, suggesting that the products of GCD1, GCD12 and GCN3 interact to affect both translational control of GCN4 and an essential function needed for entry into the cell cycle. We have completed the DNA sequence of GCN3 and have begun the preparation of GCN3-specific antisera to be used to examine the cellular location of GCN3 and to probe for interactions between GCN3 and other regulatory proteins.





## LABORATORY OF NEUROCHEMISTRY AND NEUROIMMUNOLOGY

- |                 |   |
|-----------------|---|
| Z01 HD 00056-11 | Biosynthesis, Processing & Secretion of Neuropeptides<br>& Pituitary Peptide Hormones<br>Yoke Peng Loh, Ph.D. |
| Z01 HD 00058-11 | Peptides in the Adult and Developing Vertebrate<br>Nervous System<br>Harold Gainer, Ph.D.                     |
| Z01 HD 00705-05 | Functional Organization of the Nerve Terminal<br>Harold Gainer, Ph.D.   |
| Z01 HD 01200-01 | Molecular Mechanisms in Neuronal Structure and Function<br>Harold Gainer, Ph.D.                               |



NICHD ANNUAL REPORT  
October 1, 1985 to September 30, 1986

Laboratory of Neurochemistry and Neuroimmunology

This laboratory is concerned with the development, functional organization and interactions between three major integrative systems in the body - the central nervous system, endocrine system, and the immunological system. The approach of the laboratory is cell biological in nature, and hence utilizes a wide variety of techniques and concepts from a number of disciplines, e.g., physiology, biochemistry, morphology, immunology, and molecular biology. In particular, we study various secretory peptides, intracellular membrane systems, and cytoskeletal proteins which are found in these organ systems and which are essential to their functions (i.e., peptide biosynthesis and regulation, neuronal morphology and function, etc.). A special emphasis is placed on the study of the cellular development of these organ systems.

The activities of the laboratory are divided into two sections and one unit.

1. Section on Functional Neurochemistry

A large number of neuropeptides have been identified as chemical transmitters of information in the nervous system. These known peptides (and presumably those still to be discovered) act as conventional neurotransmitters in a synaptic, paracrine, or autocrine fashion, as well as in neuroendocrine systems. In addition to the well-known hypophysiotrophic regulatory peptide hormones (e.g., CRF, LHRH, TRH, etc.), there are neuropeptides involved in a wide variety of other CNS functions (e.g., pain, blood pressure control, memory, etc.) This Section's goal is to study the cell biology of peptidergic neurons in the context of their regulatory functions in the nervous system. In particular, we specifically study the expression of neuropeptides during CNS development and their impact on the development of organismic functions and morphology.

Our studies have been focused on two specific peptidergic systems in the hypothalamus, the oxytocinergic and vasopressinergic neuronal systems, and the LHRH neuronal system. We chose to concentrate on these systems because the neurons that constitute them represent excellent models of peptidergic neurons in the nervous system. The hypothalamo-neurohypophysial system is studied because the neurons that constitute it (i.e., the oxytocin and vasopressin magnocellular neurons) are prototypic of peptidergic neurons in the central nervous system. These neurons populate two defined topographic sites in the brain (the paraventricular and supraoptic nuclei), and have a specific axonal pathway and termination site, all of which are accessible to experimental manipulation in vivo by stereotaxic, morphological, and biochemical-pharmacological techniques. Access to all three of the critical neuronal structures, i.e., the cell body, the axon, and the terminal in the hypothalamus, median eminence, posterior pituitary, respectively, permits a cell biological analysis of this system. The cell bodies in the hypothalamus are responsible for the biosynthesis of the vasopressin (AVP) and oxytocin (OT) prohormones and peptides. In addition to examining these neurons in the mammal (rat and mouse), we have extended these studies to the neurons that synthesize the evolutionarily related vasotocin



(AVT) and mesotocin (MT) peptides in the frog (Xenopus laevis). A second peptidergic system under study in the laboratory is the LHRH system in the rat. The LHRH neurons found in the hypothalamus project principally to the median eminence. The secretion of LHRH into the portal blood system regulates the development and function of reproductive system. In addition, to being the objects of cell biological studies, the embryonic development of these two neuronal systems is currently also under intensive study. A third peptidergic neuronal system found in the hypothalamus is also currently under study, i.e., the corticotropin releasing factor (CRF) system. This system projects to the external zone of the median eminence, where the secretion of CRF into the portal system causes the release of ACTH from corticotropes in the anterior pituitary. We are particularly focusing on the co-existence of AVP in the CRF neurons, and the modulation of AVP biosynthesis and release during adrenalectomy and stress.

In addition to the above studies on peptidergic neurons, our Section has embarked on an analysis of macromolecular involvement in neuronal structure and recognition. Three model systems are being used for this purpose; the Xenopus developing nervous system, the squid giant axon, and the mouse hypothalamo-neurohypophysial system. Each of these systems makes a unique contribution to the study of neuronal structure and development (see below). The progress in each of the above projects will be described below in relation to the individuals who are the principal experimenters. The ability to make monoclonal antibodies (Mabs) against small quantities of antigens continues to be an important aim of our laboratory. Ms. Shirley House, a technician in our laboratory, has been studying how to optimize in vitro immunization protocols for this purpose. She has done extensive analyses of the efficacy of a variety of conditioning media (i.e., from mouse thymus, cow thymus, and EL-4 cells) to promote effective in vitro immunization. At present she is employing these techniques, in collaboration with the UNSS, to produce Mabs against calcium binding proteins (e.g., calsequestrin). Preliminary indications are that more than 21 producing clones are being harvested by these procedures. Ms. Sharon Key, another technician in our Section, is developing methods to use these Mabs and antisera in immunocytochemical procedures at the light and electron microscopic levels. These procedures, which include pre- and post-embedding staining, silver intensification, staining of tissue cultured, whole mounts, vibratome sections, etc., are ultimately used by all members of the Section in their research. At present, Ms. Key has successfully mastered these techniques and is educating various members in our laboratory on their use.

Progress in the analysis of the peptidergic neuronal systems is as follows: Dr. M.H. Whitnall has extensively studied the response of the CRF system to adrenalectomy. He has demonstrated that: 1) The CRF neurons are composed (in normal rats) of two populations, one which contains AVP in co-existence with CRF in its secretory vesicles (50%) and one which only contains CRF (50%), 2) This co-existence phenomenon has been demonstrated by using 3 antibodies against the three components of the AVP prohormone (AVP, NP-VP, and the glycopeptide), in itself raising the ultrastructural analysis of co-existence to a new level of comprehensiveness, 3) He has shown that following adrenalectomy there is only one population, i.e., the co-existent one, thereby demonstrating a neuronal plasticity relative to function in this system. These studies were done by serial section immunocytochemistry and quantitative analysis. Dr. M. Altstein has studied the development of the AVP and OT neuronal system by quan-

titative RIA methods, and has shown that AVP precedes OT expression by 6 days in embryonic development, and that OT expression is a post-natal phenomenon. In addition, she has evaluated the cause of this differential development and found that both AVP and OT prohormones are expressed concurrently but that the OT processing is delayed. Dr. Altstein has also discovered that in vivo the first processing endopeptidase cleaves on the carboxyl-side of the Arg in the Lys-Arg signal in the OT-prohormone. Dr. K. Conway has analyzed the distribution of over 4000 AVP and MT neurons in the adult Xenopus brain, and has analyzed the crossreactivities of mammalian Np Mabs in these neurons. He has discovered a fascinating epitope switch in the frog Np versus the rat Np, which could be explained by a switch in exon C in the mammalian versus frog gene. His main objective to examine the lineages of the AVT and MT neurons in Xenopus, is now in progress. Dr. S. Wray has examined LHRH neuron development in the rat from the neonate (2 days pn) to adulthood. Her observations are that smooth LHRH cells are transformed to irregular LHRH cells during puberty, and that this occurs independently of gonadal steroid feedback. Current efforts are being directed at determining whether specific synaptic inputs to the LHRH cells are modified during this dramatic morphological change. This involves the use of double-label E.M. immunocytochemistry. In addition, Dr. Wray has developed an in vitro organotypic culture of the LHRH system, and has succeeded in growing substantial numbers of LHRH neurons for as long as one month in vitro. Of even greater significance, she has been able to co-culture brain stem, hypothalamus, and pituitary successfully so as to allow a study of these three-dimensional components in the brain in a two-dimensional space. She is currently studying trophic relations in these co-cultures.

Progress in the neuronal structure program is as follows: Dr. B. Szaro has completed his analysis of Mabs against Xenopus neuronal cytoskeleton, and is currently using these characterized Mabs to evaluate the expression of cytoskeleton elements during nervous system development. Some of these brain specific Mabs (e.g., MAP-1, neurofilaments, etc.) are recognizing specific cells and fibers in the nervous system as early as stage 33 in development, and efforts are currently under way to move to the neural plate stage. Dr. L. Charnas is currently screening a Xenopus brain cDNA library with intermediate filament probes, and several promising clones are under study. Both of these approaches (molecular and immunological) are directed at understanding the development of neuronal processes (e.g., axons and dendrites) in Xenopus. Dr. H. Gainer's studies in the squid giant axon system on neurofilament structure and function continue to support the hypothesis that the genesis of neurofilament structure is determined topographically in the neuron, i.e., biosynthesis and assembly in the cell body, phosphorylation in the axon, and proteolysis in the terminal. This topographic organization of regulation of post-translational modification is currently under study with regard to mechanism. A new program on cellular recognition in the nervous system, using the hypothalamo-neurohypophysial system, has been initiated. Dr. Y. Hara is currently attempting to isolate the AVP and OT genes in the mouse from a mouse genomic library, using human AVP and OT gene probes. The aim of this is to isolate the mouse 5'-flanking (promoter) sequences, so as to use these promoter regions in the transgenic mouse model. Given AVP or OT promoters linked to other structural genes (e.g., class I MHC, NCAM, etc.), we can theoretically study the impact of expression of virtually any macromolecules on AVP and OT neuronal development. Dr. A. Nieburgs is beginning a project on the relationship of posterior pituitary astrocytes (i.e., pituicytes, glia) on nerve terminal development in the posterior pitui-



tary.

## II. Section on Cellular Neurobiology

The research goal of this Section is to study brain and pituitary peptides which are involved in intercellular neurocommunication and fetal development.

The emphasis has been on the ACTH/endorphin/ $\alpha$ -MSH family of peptides. Endorphin is an opiate peptide that is found in brain, pituitary and placenta.  $\alpha$ -MSH is present in brain and pituitary, but in humans it is present in the pituitary only during pregnancy and in the fetus. This peptide has been implicated to have an effect on fetal growth and development. ACTH is a pituitary peptide which is traditionally known to stimulate steroidogenesis. However, it is also found in brain. All these peptides have been shown to have central nervous system effects and are thought to act as neurotransmitters and neuromodulators. The major focus has been to continue to study the enzymology and regulation of biosynthesis, packaging and secretion of this family of peptides. Within the past year two interrelated projects have been pursued.

The ACTH,  $\alpha$ -MSH and endorphin peptides are synthesized in the intermediate lobe of the pituitary from a common, glycoprotein prohormone (pro-opiomelanocortin, POMC) of about 32,000 daltons in size. We have assayed for several enzymes involved in the processing of this prohormone. These include a carboxypeptidase B-like enzyme, an aminopeptidase B-like enzyme and a paired basic residue-specific prohormone converting enzyme (PCE). This latter enzyme has been purified to apparent homogeneity from secretory vesicles of the bovine pituitary intermediate lobe and neural lobe by Drs. Parish, Tuteja and Loh. PCE from both lobes appear to have very similar characteristics and are likely to be the same enzyme. PCE is a glycoprotein, has a molecular weight of ~70,000 daltons and cleaves several precursors (POMC, pro-vasopressin, pro-insulin and pro-enkephalin) at paired basic residues to yield products seen in the tissues that synthesize these prohormones or neuropeptide precursors. The enzyme has a pH optimum of 4.0-4.5 consistent with intravesicular pH. Recently using a simpler substrate  $\beta$ -lipotropin ( $\beta$ -LPH), the C-terminal part of POMC, we have determined the  $K_m$  and  $V_{max}$  of the cleavage of the Lys37-Lys38 and Lys57-Arg58 of  $\beta$ -LPH to be 1.9  $\mu$ M and 4.6 nmol/ $\mu$ g protein/h; and 2.5  $\mu$ M and 9.1 nmol/ $\mu$ g protein/h, respectively. Inhibitor studies have shown that PCE is inhibited by two aspartyl protease inhibitors, pepstatin A and diazoacetyl-norleucine methyl ester, but not by thiol or serine protease inhibitors. Thus PCE is the first aspartyl protease isolated in mammalian cells that have specificity towards basic residues. Further evidence that PCE is a physiologically significant enzyme in prohormone processing comes from recent studies showing that the enzyme was secreted from dissociated bovine intermediate lobe cell together with the hormone,  $\alpha$ -MSH, and the release of both were inhibited by dopamine. Finally, we have demonstrated that POMC processing was inhibited by pepstatin A in intact mouse neurointermediate lobes. This latter result shows that PCE has fulfilled the most stringent criteria for the identification of a physiologically relevant prohormone processing enzyme as set forth by Docherty and Steiner (Ann. Rev. Physiol. 1982) i.e., an inhibitor of the putative enzyme must interfere with the precursor processing in the intact cells. PCE is the first enzyme demonstrated to process intact prohormones and is of potential commercial value for the production of peptide hormones and neuropeptides when used for the limited cleavage of precursors synthesized by bacteria which has been transfected with



a vector carrying the prohormone cDNA sequence. A patent for PCE has been filed. We have also purified sufficient PCE for amino acid sequence analysis and our goal in the coming year is to clone the enzyme.

Dr. Nigel Birch is making good progress in the purification of the amino peptidase B-like enzyme from pituitary secretory vesicles. He has shown that the enzyme cleaves an Arg at least 6-fold faster than a Lys at the N-terminal of a peptide. This result is exciting since it can explain the accumulation of high levels of Lys- $\gamma$ -MSH in the intermediate pituitary, but no other Arg extended POMC derived peptides, except when the Arg is followed by a Pro, as in CLIP. In this case the aminopeptidase B-like enzyme will not cleave the Arg.

Progress has also been made on the study concerning the regulation of synthesis of pro-opiomelanocortin (POMC) in the toad intermediate lobe (IL). Using organ cultured toad neurointermediate lobes, B. Myers has shown that dopamine effectively down regulated the biosynthesis of POMC. The dopamine receptor in the toad intermediate lobe was pharmacologically characterized as being in the D2 category and negatively coupled to adenylate cyclase. Presence of dopamine in the culture medium resulted in a decrease in intracellular cAMP and POMC synthesis in the intermediate lobe. This decrease in POMC synthesis was prevented by the addition of 8-Bromo-cAMP to the medium. B. Myers has also examined the regulation of POMC synthesis at the transcriptional level during black and white background adaptation of the frog. Using a 48 mer synthetic probe to frog POMC and quantitative in situ hybridization technology, she has shown that the POMC mRNA level in the IL was greatly increased during black background adaptation.

This year Dr. Stella Elkabes has initiated a study on the regulation of POMC synthesis in the intermediate and anterior pituitary of mice that have been subjected to hyperosmotic stress by supplying the animals with 2% saline in place of drinking water. Salt loading for two days resulted in a 175% increase in POMC mRNA levels in the anterior pituitary. POMC synthesis and processing was also enhanced in this tissue. In contrast, POMC mRNA levels and synthesis decreased by 50% in the intermediate lobe. Neuroendocrinological components (such as CRF and vasopressin) which may regulate these changes in POMC synthesis are currently being studied.

### III. Unit on Neuronal Secretory Systems

The nerve terminal is a highly specialized region of a neuron separated from the neuronal soma by an axon, whose function is to release neurotransmitter substances when stimulated by an electrical signal carried by the axon. Thus the nerve terminal plays the central role in the nervous system function that operates by signal transmission between cells, by means of secretion of neurotransmitters. Modulation of the quantity of the transmitter released at the terminal may form the basis for all central nervous system functions, including integration of information, long term information storage, and retrieval. Because of this complexity (cellular heterogeneity, and their complex organization), a basic understanding of the molecular mechanisms of nerve terminal function in the central nervous system is still lacking.

The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. The hypothalamo-neurohypophysial sys-

stem represents model central nervous system neurons, because of their homogeneity and discrete localization, which are eminently accessible for experimentation. The nerve terminals of these neurons are discretely localized in the neurohypophysis, where they are accessible to experimental manipulations both in vivo and in vitro. These nerve terminals can be isolated from the neurohypophyses without contamination by the post-synaptic membrane, unlike nerve terminals from other regions in the central nervous system.

Studies on the elucidation of the functional organization of the nerve terminal forms the central theme of the Unit on Neuronal Secretory Systems. A preparation of neurosecretosomes (nerve endings from the neurosecretory neurons) has been obtained. This preparation is highly enriched in nerve terminals and forms the basic experimental model. Four different aspects of nerve terminal function are under investigation. Firstly, the mechanisms of  $\text{Ca}^{2+}$  ion homeostasis in the nerve terminal is being investigated both from the membrane transport standpoint and from the point of binding to intraterminal  $\text{Ca}^{2+}$  binding protein. Secondly, the mechanism of secretory regulation at the nerve terminals is being studied with a view to understanding the molecular mechanisms of facilitation and to characterize the physiological role of other peptide receptors on the nerve terminals in the regulation of secretion. Thirdly, studies on the transport processes in neurosecretory vesicles continue. Experiments are underway to conclusively identify the proton pumping  $\text{Mg}^{2+}$  ATPase on neurosecretory vesicles and compare its properties with the mitochondrial proton translocator. The neurosecretory vesicles are also studied to elucidate the presence of ionic channels intrinsic to this membrane. This study is carried out in collaboration with Drs. Gerald Ehrenstein and Stanley Elis of Laboratory of Biophysics, NINCDS. Fourthly, in collaboration with Dr. Ian Forsythe of Laboratory of Developmental Neurobiology, experiments are underway to characterize membrane ionic channels on the nerve terminals using patch clamp techniques.

Dr. Carolyn Bondy is involved in studies on elucidating the cellular mechanisms involved in frequency-dependent facilitation of vasopressin secretion from isolated neurohypophyses. Preliminary experiments suggest that  $\text{K}^{+}$  channels are important in facilitation of secretion. Dr. Bondy also has developed immobilized neurosecretosome model to study the kinetics of secretion from nerve terminals in response to depolarizing stimuli.

Dr. James Garbern has focussed on studies on  $\text{Ca}^{2+}$ -binding proteins present in nerve terminals. He has detected two high affinity  $\text{Ca}^{2+}$ -binding proteins which are unique to nerve terminals and is in the process of purifying them for characterization.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00056-11 LNN

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis, processing &amp; secretion of neuropeptides &amp; pituitary peptide hormones

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Y. P. Loh	Head	LNN, NICHD
Others:	David Parish	Visiting Fellow	LNN, NICHD
	Renu Tuteja	Visiting Fellow	LNN, NICHD
	Nigel Birch	Visiting Fellow	LNN, NICHD
	Stela Elkabes	Visiting Fellow	LNN, NICHD
	Maria Castro	Visiting Fellow	LNN, NICHD
	Baldwin Wong	Microbiologist	LNN, NICHD
	Brenda Myers	Junior Fellow	LNN, NICHD

## COOPERATING UNITS (if any)

Tom Zoeller, Michael Brownstein and Hirato Okayama, LCB, NIMH; Eugene Butler, LDN, NICHD; Kathy Zoon (FDA)

## LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

## SECTION

Section on Cellular Neurobiology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

## TOTAL MAN-YEARS:

6.75

## PROFESSIONAL:

5.0

## OTHER:

1.75

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

The biosynthesis of ACTH, endorphin,  $\alpha$ -MSH, vasopressin and oxytocin, was studied, with emphasis on the enzymes involved in the proteolytic processing of the respective prohormones. A prohormone converting enzyme (PCE) which specifically cleaves at paired basic residues of the endogenous prohormones (pro-opiomelanocortin, pro-oxytocin and pro-vasopressin) to the active hormones, has been purified from bovine pituitary intermediate and neural lobe secretory vesicles. Purified PCE from both lobes appears to be identical and has been characterized as a ~70,000 dalton glycoprotein. PCE exists in a soluble and membrane associated form and was found to cleave pro-insulin to insulin as well. A carboxypeptidase B-like enzyme and an aminopeptidase B-like enzyme which function to remove the basic residues from the C- and N-terminal, respectively, from the peptide hormone, following the action of PCE, have been found in intermediate lobe and neural lobe secretory vesicles. The aminopeptidase B-like enzyme has been partially purified and characterized as a >75,000 dalton metalloproteinase. The regulation of biosynthesis of pro-opiomelanocortin (POMC) by dopamine and cAMP was studied in the toad pituitary intermediate lobe. Pharmacological analyses indicate that the toad intermediate lobe dopamine receptor is of the D<sub>2</sub> category and negatively coupled to adenylate cyclase. Thus the dopamine acts, subsequent to binding to the receptor, by lowering the intracellular cAMP level which then results in a decrease in POMC synthesis in the tissue. The regulation of POMC biosynthesis was also studied in mice under hyperosmotic stress. Salt loading the animals for two days resulted in a 2-3 fold increase in POMC mRNA levels, POMC synthesis and processing and secretion of POMC derived peptides from the anterior pituitary. In contrast POMC mRNA levels, POMC synthesis and secretion of POMC derived peptides were decreased by 56% in the intermediate lobe.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00058-11 LNN
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Peptides in the adult and developing vertebrate nervous system		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Harold Gainer Head LNN, NICHD  Others: Mark Whitnall Senior Staff Fellow LNN, NICHD Kevin Conway Staff Fellow LNN, NICHD Susan Wray PRAT Fellow LNN, NICHD Miriam Altstein Weizmann Inst. Fellow LNN, NICHD Sharon Key Biologist LNN, NICHD		
COOPERATING UNITS (if any) Dr. B. Gahwiler, Sandoz Ltd., Switzerland; Dr. D. Smyth, MRC, London; M. Castel, Hebrew University		
LAB/BRANCH Laboratory of Neurochemistry and Neuroimmunology		
SECTION Section on Functional Neurochemistry		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland		
TOTAL MAN-YEARS 6.0	PROFESSIONAL: 5.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Analysis of the <u>development</u> of <u>vasopressin</u> and <u>oxytocin</u> neurons by immunocytochemical and RIA procedures have shown that there is a profound differential expression of peptides during <u>fetal development</u> . The vasopressin (AVP) neurons are more precocious than oxytocin (OT) neurons during development in extent of peptide expression and fiber outgrowth. This difference in peptide expression is due to an apparent absence of <u>carboxypeptidase-B-like processing activity</u> in the OT cells. The <u>co-existence</u> of AVP and CRF in nerve terminals in the external zone of the median eminence was conclusively demonstrated by serial section <u>immunocytochemistry</u> at the ultrastructural level, using antibodies against all three components of the AVP prohormone. Co-existence was found at the <u>secretory vesicle level</u> , implying co-secretion of both CRF and AVP from a specific subpopulation of nerve endings. Vasotocin (AVT) and mesotocin (MT) neurons have been mapped in 3-dimensions in adult <u>Xenopus</u> hypothalamus, and neurophysin immunoreactivities have been documented. Expression of these peptides appears at least at stage 45 in <u>Xenopus</u> development. An <u>organotypic tissue culture model</u> of rat LHRH neurons has been developed, and preliminary data suggests <u>trophic interactions</u> between brain stem, hypothalamus, and pituitary regions <u>in vitro</u> .		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00705-05 LNN

PERIOD COVERED  
October 1, 1985 to September 30, 1986TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Functional organization of the nerve terminal

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dr. James Russell Head, Unit on Neuronal Secretory Systems LNN, NICHD

Others: Carolyn Bondy Medical Staff Fellow LNN, NICHD  
James Garbern Medical Staff Fellow LNN, NICHD

COOPERATING UNITS (if any)

Gerald Ehrenstein, LB, NINCDS; Stanely Ellis, LB, NINCDS; Ian Forsythe, LDN, NICHD;  
Harold Gainer, LNN, NICHD

LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

SECTION

Unit on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

3.5

PROFESSIONAL:

3.0

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. A preparation of highly purified neurosecretosomes (nerve endings from the neurosecretory neurons) was developed. This preparation forms the unique experimental system to study various aspects of nerve terminal function. Membrane systems obtained by disruption of the neurosecretosomes were fractionated and ATP-dependent  $\text{Ca}^{2+}$  accumulating membranes were identified. Furthermore, permeabilizing the plasma membrane by the use of digitonin or saponin has provided access to study intra-terminal  $\text{Ca}^{2+}$  accumulating systems. The neurosecretosomes have been successfully immobilized onto solid matrices so that the kinetics of  $\text{Ca}^{2+}$ -dependent secretion can be monitored. The presence of opiate receptors on the nerve ending membranes was identified and characterized. The kappa type opiate receptor is predominantly present on neurosecretory nerve endings. Studies on isolated neurosecretory vesicles have led to the identification of both a  $\text{Ca}^{2+}$ -activated cation channel of large conductance level ( $>400\text{Ps}$ ) and an anion channel. These channels may be important in the mechanism of exocytotic secretion at the nerve terminal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01200-01 LNN
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Mechanisms in Neuronal Structure and Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Harold Gainer	Chief LNN, NICHD
Others:	Ben Szaro	Staff Fellow LNN, NICHD
	Shirley House	Biologist LNN, NICHD
	Yoshinobu Hara	Visiting Fellow LNN, NICHD
	Larry Charnas	NRSA Fellow LNN, NICHD
	Andra Nieburgs	NIH Extramural Fellow LNN, NICHD
COOPERATING UNITS (if any) Dr. H. Pant, NIAADA; Dr. R. Cohen, Univ. of Illinois; Dr. C. Klee, NCI; Dr. K. Ozato, NICHD		
LAB/BRANCH Laboratory of Neurochemistry and Neuroimmunology		
SECTION Section on Functional Neurochemistry		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )  Analyses of <u>cytoskeletal proteins</u> in the <u>Xenopus</u> and <u>squid nervous system</u> has been facilitated by the production of a panel of <u>monoclonal antibodies (Mabs)</u> against these proteins. In the squid, these Mabs have been used to show that <u>phosphorylation of neurofilaments</u> occurs only in the <u>axon</u> . Analysis of the <u>protein kinase-calcium activated protease-neurofilament complex</u> in the squid giant axon has shown that the protein kinase is similar to type II casein kinase, and that an inhibitor of this kinase is located in the cell bodies in the stellate ganglion. In the <u>Xenopus nervous system</u> the Mabs have allowed for the detection of neuronal-specific cytoskeletal proteins (including neurofilaments) during embryonic development. A <u>Xenopus brain cDNA library</u> is being probed for clones containing neurofilament sequences. A <u>mouse genomic library</u> is being probed for the presence of <u>vasopressin</u> and <u>oxytocin precursor</u> sequences.		



LABORATORY OF THEORETICAL AND PHYSICAL BIOLOGY

- Z01 HD 00040-11     Statistical and Mathematical Studies and Molecular  
                                 Interactions  
                                 Peter J. Munson
- Z01 HD 00165-11     Isolation and Characterization of Macromolecular  
                                 and Cellular Particles  
                                 Andreas Chrambach, Ph.D.
- Z01 HD 00171-10     Electrophoretic Methodology  
                                 Andreas Chrambach, Ph.D.
- Z01 HD 00189-05     Computer Programs for Analysis of Laboratory and  
                                 Clinical Data  
                                 David Rodbard, M.D.
- Z01 HD 01400-04     Clinical Applications of Stable Isotopes  
                                 Alfred L. Yergey, Ph.D.
- Z01 HD 01401-04     Biological Applications of Thermospray Liquid  
                                 Chromatography/Mass Spectrometry  
                                 Alfred L. Yergey, Ph.D.
- Z01 HD 01404-03     Characterization of Opioid Receptors in Brain and  
                                 Peripheral Tissues  
                                 David Rodbard, M.D.
- Z01 HD 01405-02     Computer Programs to Aid Intensive Insulin Therapy  
                                 for Type-I Diabetes Mellitus  
                                 David Rodbard, M.D.



NICHD ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Laboratory of Theoretical and Physical Biology

The LTPB have made several important contributions during the past year, including:

Demonstration and characterization of at least 3 types of oxytocin and vasopressin throughout the male genital tract. Oxytocin receptors are most concentrated in testis of the prepubertal pig, and decrease progressively in epididymis and vas deferens, whereas the reverse pattern of localization was observed for the  $V_1$  vasopressin receptor. A pharmacologically distinct type of receptor, designated  $V_3$  is present at extremely high concentrations in seminal vesicle, is coupled to adenylate cyclase, and resembles the vasopressin receptor of anterior pituitary. These receptors imply a physiological role for oxytocin and vasopressin in male reproduction. We have demonstrated the presence of 3 distinct subtypes of kappa receptors in bovine adrenal medulla, and are studying the effects of opiates on the regulation of catecholamine release. We have demonstrated the presence of both high and low affinity opioid receptors in neurosecretosomes from bovine posterior pituitary (in collaboration with LNN), and have shown that these are exclusively of the kappa type. The influence of saturated and unsaturated fatty acids on binding of opioids, ouabain, and beta adrenergic ligands to their receptors was also studied, to evaluate specificity of effects.

A new, universally applicable method for curve-fitting has been developed which combines the advantages of empirical methods (simplicity, versatility, flexibility, no need to provide a model or equation), with several of the advantages of mathematical modelling (objectivity, statistical hypothesis testing, standard errors of critical parameters, computerization and automation). This method promises to have widespread utility in scientific data analysis.

Objective statistical methods for detection and characterization of episodic pulsatile hormone release have been developed, and applied to a wide range of endocrine systems. When instantaneous hormone secretion is computed, it appears that > 90% of the "tonic" secretion of luteinizing hormone (LH) in man occurs in bursts lasting less than 10% of the time. This will have considerable impact on our understanding of the physiology, not only of LH, but also of many other peptide and steroid hormonal systems.

Improved methods have been developed for analysis of the "ambulatory glucose profile" for patients with insulin dependent diabetes mellitus. This involved development of novel statistical methods. These analyses are useful to the physician, diabetes educator, and the patient.

A novel statistical analysis of an SV-40 based DNA damage/repair system was used to compare several explanations of the DNA repair process (in collaboration with OSD). Results showed that insertion of one base (adenine) occurs with higher probability across from damage in the template DNA strand, regardless of the identity of the original base at that location.



A new statistical test and computer program was developed for evaluating the "goodness-of-fit" of a mathematical model to data, based on a mathematical characterization of random noise as "rough" while the signal must be "smooth."

The Section on Macromolecular Analysis has made progress in development of a method for steady-state isoelectric focusing and two-dimensional macromolecular mapping. Combined use of Immobiline pH gradients and soluble carrier ampholytes provides pH-gradient stability with improved entry of proteins into the gel and both uniformity of conductance and voltage gradient. The procedure has been streamlined so that it is now practical.

Agarose gels and highly crosslinked polyacrylamide gels have been applied to the fractionation and size-charge characterization of viruses. The "Ferguson plots" of  $\log(\text{mobility})$  versus gel concentration are nonlinear and complex: mathematical modelling has been used to derive apparent properties of the gel, and to permit reliable estimation of particle size (radius) and free mobility. In addition, slope changes in the Ferguson plot were used as a measure of the malleability of the particle. Analysis of the physical properties of viruses and cellular organelles was founded, for the first time, on commercially available standards (polystyrene microspheres with diameters determined by electron microscopy) by which the pore size and other properties of the gels were calibrated.

The Metabolic Analysis and Mass Spectrometry Section has pioneered the use of thermo-spray-liquid chromatography-mass spectrometry. Within the past year, new methods have been developed for cortisol, its metabolites and biosynthetic precursors, as well as testosterone, vitamin D, glucose, fructose, sorbitol, and acetylcholine. These methods have been applied clinically to the study of gly-cogen storage diseases, Cushings' syndrome, and hormonal changes during puberty, and other investigations (in collaboration with HGB and DEB). A new metabolite of cortisol, 11-hydroxyandrostenediol, has been discovered. This steroid is produced by an enzyme found in erythrocytes, and may be responsible for Cushings' syndrome in the presence of normal levels of cortisol.

Thermal ionization mass spectrometry studies of calcium metabolism in man are progressing. Studies are underway in patients during pregnancy and lactation. A new method for measurement of gastrointestinal calcium adsorption in the human neonate has been developed, and is being applied to the evaluation of the optimal levels of vitamin D in the nutrition of premature infants.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00040-11 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical and Mathematical Studies of Molecular Interactions

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter J. Munson	Mathematical Statistician	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Cruciani	Visiting Fellow	LTPB, NICHD
	G. Pesce	Guest Researcher	LTPB, NICHD
	D. Lichtstein	Visiting Scientist	LTPB, NICHD
	R. Jernigan	Guest Researcher	LTPB, NICHD

## COOPERATING UNITS (if any)

VCBS, NICHD (K. Dixon)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

1.0

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use stenderd unreduced type Do not exceed the space provided.)

Nonlinear least squares curve fitting techniques were applied to the modeling of multiple receptor-multiple ligand interactions, especially in characterization, subtypes of the mu receptor in rat brain and of kappa in the borvine adrenal medulla. Statistical optimal design of experiments was utilized to reduce the amounts of biological material needed. Computer programs to implement these techniques were further developed and distributed.

Statistical investigation of UV-induced DNA damage mechanisms established a probabilistic model for repair of DNA sequences.

A new statistical test for non-randomness was developed, based on intuitive concept of smoothness. This test is applicable in the analysis of regression residual.

A new, generally useful technique for comparing families of curves was developed which is not based on a fixed equation or model, but takes its shape from the curves directly. It can be utilized in bioassay, RIA, or any physiological dose-response type experiment.

A new technique for generating time-dependent normal ranges was developed for use in the 24-hour glucose profile. This method is based on a generalization of kernel smoothing approaches and non-parametric density estimation techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00165-11 LTPB															
PERIOD COVERED October 1, 1985 to September 30, 1986																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders ) Isolation and Characterization of Macromolecular and Cellular Particles																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: A. Chrambach</td> <td style="width: 33%;">Section Chief</td> <td style="width: 33%;">LTPB, NICHD</td> </tr> <tr> <td colspan="3" style="padding-top: 10px;">Others: D. Tietz</td> </tr> <tr> <td>E. Gombocz</td> <td>Visiting Fellow</td> <td>LTPB, NICHD</td> </tr> <tr> <td>L. Orban</td> <td>Courtesy Associate</td> <td>LTPB, NICHD</td> </tr> <tr> <td></td> <td>Visiting Fellow</td> <td>LTPB, NICHD</td> </tr> </table>			PI: A. Chrambach	Section Chief	LTPB, NICHD	Others: D. Tietz			E. Gombocz	Visiting Fellow	LTPB, NICHD	L. Orban	Courtesy Associate	LTPB, NICHD		Visiting Fellow	LTPB, NICHD
PI: A. Chrambach	Section Chief	LTPB, NICHD															
Others: D. Tietz																	
E. Gombocz	Visiting Fellow	LTPB, NICHD															
L. Orban	Courtesy Associate	LTPB, NICHD															
	Visiting Fellow	LTPB, NICHD															
COOPERATING UNITS (if any) USDA, Beltsville, MD (S.S. Hurtt); University of Texas, San Antonio, Texas (P. Serwer).																	
LAB/BRANCH Laboratory of Theoretical and Physical Biology																	
SECTION Section on Macromolecular Analysis																	
INSTITUTE AND LOCATION NICHHD, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS 1.5	PROFESSIONAL: 1.5	OTHER: 0															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews								
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither															
<input type="checkbox"/> (a1) Minors																	
<input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p>(1) The particle radii of the 3 plant viruses, turnip crinkle virus (TCV), hibiscus chlorotic ringspot virus (HCRSV) and pelargonium flowerbreak virus (PFBV) were determined by agarose gel electrophoresis in the presence and absence of 5 mM calcium as 12.8, 20.3, 18.3 and 29.4, 24.2 and 22.1 nm respectively. This size determination uses the entire nonlinear Ferguson plot in conjunction with computer simulation, and commercially available polystyrene microsphere size standards in the size range 20 to 60 nm. (2) Ferguson plots of bacteriophages, plant viruses and polystyrene sulfate particles with two or more points of inflection are interpreted in terms of a variation of particle size (compressibility) as a function of gel concentration. This particle size effect is superimposed on the variation of gel fiber dimension in causing the convex-sigmoidal curvature. These plots also serve as characteristic "finger prints" for the particle. (3) Ferguson plots of plant viruses from crude extracts are presently attempted in order to demonstrate pattern identity with purified virus, absence of perturbation by subcellular particles or nucleic acids, and readiness to attack clinical virus separation and identification problems.</p>																	



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00171-10 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Electrophoretic Methodology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	J.S. Fawcett	Visiting Scientist	LTPB, NICHD
	D. Tietz	Visiting Fellow	LTPB, NICHD
	E. Gombocz	Courtesy Associate	LTPB, NICHD
	L. Orban	Visiting Fellow	LTPB, NICHD
	M. Buttermann	Guest Worker (Summer Student)	LTPB, NICHD

## COOPERATING UNITS (if any)

USDA, Beltsville, MD (S.S. Hurtt); University of Texas, San Antonio, Texas (P. Serwer).

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Macromolecular Analysis

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.50

## PROFESSIONAL:

2.25

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Constancy of protein zone positions with focusing time on immobilized pH gradient gels in the pH-range 4-10 was demonstrated. (2) The procedure of focusing with Immobiline containing gels was streamlined by elimination of gel washing and drying to the original weight and pre-electrophoresis. Gradient formation was facilitated by pumping or mechanical 2-syringe device. (3) Pore limit electrophoresis (PLE) in polyacrylamide gradients up to 60% T was developed to serve as a 2nd dimensional size fractionation at the steady state, providing stationary coordinate pairs for spots on a 2-D gel pattern. (4) Agarose gel fiber properties (fiber radius, length per unit weight and volume) were estimated by computer simulation, using polystyrene microspheres as commercially available size standards. The extended Ogston theory was adapted for that purpose by substitution of functions for the fiber and particle properties previously considered constants. Effective gel fiber properties were shown to be a function of gel concentration and of the size of the particle passing through the gel. (5) Agarose gel electrophoretic analysis of plant viruses and polystyrene microbeads was carried out in a discontinuous (moving boundary electrophoresis) buffer system, with stacking gels operative at pH 6.5 and resolving gels operative at either pH 6.5 or 7.2, 0.03 M ionic strength, 0.01 M CHAPS, 0°C. This has the advantage of allowing for particle separation from dilute samples, and of providing uniformly concentrated starting zones for enhanced resolution. (6) Polyacrylamide gel electrophoresis on 30% Bis-crosslinked gels was applied to polystyrene particles with radii 10 - 60 nm in an attempt to obtain Ferguson plots with simplified curve shape compared to that in agarose, and thus to facilitate the physical characterization of particles. (7) Curvature of Ferguson plots on polyacrylamide gel was determined as a function of crosslinking in order to increase the accuracy of the characteristic free mobility (net charge) values of particles.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00189-05 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Computer Programs for Analysis of Laboratory and Clinical Data

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	P. Munson	Mathematical Statistician	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	J. E. A. McIntosh	Visiting Scientist	LTPB, NICHD
	C. Whitlock	Summer Aid	LTPB, NICHD

## COOPERATING UNITS (if any)

DCRT, DMB (B. Cole); Univ. of Virginia School of Medicine (J. Veldhuis);  
The Penn.School of Medicine (K. Oerter); Kantonspital, Winterthur, Switzerland  
(R. Lutz).

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This laboratory continues its tradition of developing novel computer programs for analysis of clinical and laboratory data. A new "universal" curve fitting method has been developed, which combines the advantages of empirical methods (e.g., polynomials) with those of mathematical modelling. The user does not have to provide an equation or mathematical function: The program estimates the curve shape or template automatically by analysis of families of curves. Optimal shifting and scaling factors for both dependent and independent variables are obtained by weighted non-linear least-squares curve-fitting with appropriate constraints (e.g., monotonicity, number of inflection points) and sharing of parameters. This method is useful in the context of bioassays, immunoassays, dose-response curves and response-versus-time curves in general. We have also developed multiple new approaches for analysis of episodic hormone secretion in man, experimental animals, and in in vitro perfused cell systems. These methods are statistically valid, objective, reliable, sensitive and yield new physiological information including the instantaneous rate of hormone secretion and the half-life or decay constant(s) for hormone metabolism and degradation. Other programs include improved methods for Lineweaver-Burk and Dixon-plot analysis of enzyme-substrate-inhibitor systems, radioimmunoassays, bioassays, radio-receptor assays, X-ray inactivation and dissociation studies. We have designed and developed a prototype statistical package for microcomputers using macros for popular spreadsheets. This package automatically tests relevant assumptions, assists the user in regard to selection of appropriate tests and options, and assists in regard to interpretation of results.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01400-04 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Applications of Stable Isotopes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alfred L. Yergey

Head

LTPB, NICHD

Others: Nora V. Esteban

Visiting Fellow

LTPB, NICHD

COOPERATING UNITS (if any) HGB, NICHD (J. Sidbury); Lab. of Mathematical Biology, NCI (D. Covell); Dept. Ped. Washington Univ. Med. Schl., St. Louis, MO (L. Hillman); Dept. Endocrin., Mayo Clinic (R. Eastell); Cincinnati Children's Hosp. (B. Specker)

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Unit on Metabolic Analysis

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

.5

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Studies of whole body calcium dynamics have been continued using lactating women with themselves as controls at a time at least one year from the lactation period. Simulations of the time period required to determine the first of presumably several bone exchange parameters suggests that studies be carried out for at least six weeks rather than the three used previously. (2) Determination of fractional absorption using the dual tracer technique and monitoring the tracers in urine has been studied in infants (6), adult women (14) and two siblings with end organ vitamin D resistance. Total absorption in the first two groups was virtually the same, but there is a strong suggestion of age dependent slowing in the rate of absorption. The fractional absorption of the vitamin D resistant siblings was very low (~10%), unresponsive to treatment with vitamin D or 1,25-OH<sub>2</sub>-D, and was invariant under the clinical treatment of repletion via intravenous calcium.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 HD 01401-04 LTPB
<b>PERIOD COVERED</b> October 1, 1985 to September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less Title must fit on one line between the borders.) Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	A. Yergey	Head LTPB, NICHD
Others:	D. Liberato N. Esteban	Sr. Staff Fellow Visiting Fellow LTPB, NICHD LTPB, NICHD
<b>COOPERATING UNITS</b> (if any) Div. of Ped. Met., Dept. of Ped., Duke Univ., Durham, NC (D. Millington and C. Roe); LMG, NICHD (J. Sidbury and J. Muenzer); DEB, NICHD (L. Loriaux and T. Loughlin); LDN, NICHD (D. Brenneman); NRL, Chem. Div. (R. Colton, D. Kidwell).		
<b>LAB/BRANCH</b> Laboratory of Theoretical and Physical Biology		
<b>SECTION</b> Unit on Metabolic Analysis		
<b>INSTITUTE AND LOCATION</b> NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.5	1.0	0.5
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  (1) Performed initial clinical studies for measurement of cortisol production rate (Protocol 85-CH-33) using 9,9,12-d <sub>3</sub> -cortisol as the tracer in the primed infusion technique with infusion rate equal to about 2% of maximum daily production rate. (2) Observed the presence of markedly increased levels of 11-OH-androstene dione in the serum of a normal cortisolemic but Cushingoid patient studied in the above protocol. (3) Performed clinical studies for measurement of glucose production rate (Protocol 85-CH-15) using uniformly labelled <sup>13</sup> C-glucose (98% enriched) as the tracer in the primed infusion technique. Infusion rates equalled about 0.5% of the expected residual production rate of 4 subjects with Type I Glycogen Storage Disease. (4) Quantified acetylcholine present in synaptosomes prior to studies of inhibition of release by botulism toxin.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01404-03 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	D. Lichtstein	Visiting Scientist	LTPB, NICHD
	R. Lutz	Visiting Scientist	LTPB, NICHD
	S. Schwarz	Visiting Scientist	LTPB, NICHD
	D. Maggi	Visiting Fellow	LTPB, NICHD
	R. Cruciani	Visiting Fellow	LTPB, NICHD
	A. Katki	Chemist	LTPB, NICHD
	H. Xu	Guest Researcher	LTPB, NICHD

## COOPERATING UNITS (if any)

LNN, NICHD (H. Gainer, J. Russell and M. Lang); Univ. Florence, Dept. of Endocrin., (M. Maggi); NIADDK (H. Pollard), E. Rojas; Rockefeller Univ. (P. Morris); DMNB, NINCDS (S. Kassis).

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.8

## PROFESSIONAL

2.5

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used quantitative ligand binding studies to characterize the multiple types and subtypes of opioid and other peptide receptors in rat brain, bovine adrenal medulla, bovine posterior pituitary, and other tissues. We have demonstrated the presence of receptors for oxytocin and a V1 subtype of vasopressin receptor in porcine testis, leydig cells, tunica albuginea, epididymis and vas deferens. A new, novel type of vasopressin receptor has been demonstrated in porcine seminal vesicle at concentrations in excess of those in renal medulla. Unlike the V1 receptor, these receptors are coupled to adenylate cyclase. Neurosecretosomes from bovine posterior pituitary are shown to contain high concentrations of opioid receptors, which are exclusively of the "Kappa" type: mu, delta and benzomorphan sites are not present. At least two classes of sites of differing affinities ( $K_D \approx 1$  nM,  $K_D \approx 1$   $\mu$ M) are present.

Receptors for ouabain, digoxin, and digitoxin in rat brain and cardiac muscle have been characterized, and also consist of two classes of sites, both of which are sensitive to  $K^+$ . The effects of fatty acids on the binding of these cardiac glycosides and of opioid peptides has been studied systematically.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01405-02 LTPB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	P. J. Munson	Statistician	LTPB, NICHD
	N. Esteban	Visiting Fellow	LTPB, NICHD
	N. Pernick	Guest Researcher	LTPB, NICHD
	M. Jaffe	Guest Researcher	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Victor	Guest Researcher	LTPB, NICHD

## COOPERATING UNITS (if any)

Albert Einstein College of Medicine (R. Masse, O. Langer, D. Lucido);  
University of Pittsburgh School of Medicine (A. Robinson, J. Johnston).

## LAB/BRANCH

Laboratory of Theoretical and Physical Biology

## SECTION

Section on Theoretical Biology

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.4

## PROFESSIONAL:

0.4

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed computer programs to assist physicians, diabetes educators, other paramedical personnel, and patients with intensive insulin therapy. The programs provide analysis of the ambulatory glucose profile, using manual entry and/or verified data from recording or "memory" glucose reflectance meters. Graphical and statistical displays are accompanied by detailed written interpretation, with advice and explanations for alterations of insulin dosage, timing, type, or dietary changes. Extensive human engineering and fail-safe features are provided. Nonparametric statistics and principles of exploratory data analysis are combined with techniques for transformation, weighting, smoothing and interpolating, using newly developed, original statistical methods. The programs also provide analysis of patient compliance, and have potential educational value.



OFFICE OF THE SCIENTIFIC DIRECTOR

- Z01 HD 00093-12    Mechanism of Action of Nerve Growth Factor  
                         Gordon Guroff, Ph.D.
- Z01 HD 01500-04    Adenovirus(AD) and SV40: Molecular and Cellular Biology  
                         Arthur S. Levine, M.D.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00093-12 OSD

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Mechanism of Action of Nerve Growth Factor

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI.	G. Guroff	Head	OSD:NICHD	
Others:	G. Dickens	Biol. Lab. Tech.	K. Fujita	Guest Researcher
	P. Lazarovici	Vis. Associate	S. Koizumi	Vis. Fellow
	C. Greiner	Biologist	Y. Matsuda	Guest Researcher
	T. Hama	Vis. Fellow	E. Yavin	Guest Researcher
	P. Contreras	PRAT Fellow		(Courtesy)
	Y. Mizrachi	Vis. Fellow	J. Tanner	Fed. Jr. Fellow

## COOPERATING UNITS (if any)

Department of Neurology, University of Chicago, Chicago, IL  
 Department of Neurobiology, Weizmann Institute, Rehovot, Israel  
 Department of Biochemistry, Tohoku Dental University, Koriyama, Japan

## LAB/BRANCH

Office of the Scientific Director

## SECTION

Section on Growth Factors

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

9.25

## PROFESSIONAL

7.25

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type Do not exceed the space provided.)

Nerve growth factor (NGF) is a polypeptide required for the survival and development of sympathetic and sensory neurons. It controls the expression of specific genes in these neurons. The molecular mechanism(s) by which the factor controls gene expression is not known. Our studies are focused on the intracellular events which follow the binding of NGF to its receptor in PC12, a cell which differentiates in response to NGF. One of the earliest events is a calcium-dependent activation of phosphoinositide metabolism. This finding is consistent with another of our results, namely, that NGF causes an activation of the  $Ca^{2+}$  and phospholipid-dependent enzyme protein kinase C. This activation is one of several changes in kinase function that occur, in some cases sequentially, within the cells. We have developed a cell-free, soluble phosphorylation system which reflects the prior treatment of the cells with NGF. This system involves the phosphorylation of a protein of 100,000 daltons (Nsp100). In extracts from cells treated with NGF the phosphorylation of Nsp100 is decreased. We have shown that the phosphorylation is decreased by phosphorylation of Nsp100 kinase by protein kinase C. We have prepared two other cell-free phosphorylation systems that also reflect the actions of NGF, one ribosomal and one nuclear. The pattern that is emerging is that NGF acts through a series of kinases in the cell leading to the phosphorylation of some key cellular proteins. One of these proteins is in the nucleus and its phosphorylation may underlie changes in gene expression. We have found a decreased nuclease sensitivity of DNA in NGF-treated cells, accompanied by a change in the morphology of the DNA. The morphological change correlates in time with one specific alteration in the cell phenotype, a disappearance of receptors for EGF, a mitogen for PC12 cells. Our recent studies show a decrease in the biosynthesis of the receptor. Our current effort is to explore the transcriptional regulation of the EGF receptor gene. Our hypothesis is that the decrease in the synthesis of the receptor for this mitogen is part of the mechanism by which NGF instructs the cell to differentiate.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 01500-04 OSD
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Adenovirus (Ad) and SV40: Molecular and Cellular Biology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)		
PI:	A.S. Levine	Head OSD, NICHD
Others:	C.T. Patch	Sr. Invest. N. Tuteja Vis. Fellow
	K. Dixon	Sr. Staff Fellow E. Roilides Vis. Fellow
	J.M. Hauser	Microbiologist M. Protic-Sabljić Guest Res.
	B.J. Matthews	Staff Fellow
	K. Murai	Visiting Fellow
	M.H. Haddada	Visiting Fellow
COOPERATING UNITS (if any) Laboratory of Immunopathology, NIAID (A.M. Lewis, Jr.); Dept. of Medicine, National Jewish Hospital and Research Center, Denver (J. Cook); Laboratory of Theoretical and Physical Biology, NICHD (P. Munson); Laboratory of Developmental Pharmacology, NICHD (J. Gielen).		
LAB/BRANCH Office of the Scientific Director		
SECTION Section on Viruses and Cellular Biology		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
8.0	7.0	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided ) Understanding the mechanisms of regulation of cellular proliferation and differentiation is basic to understanding development of multicellular organisms. One approach to investigating these regulatory mechanisms is to study the behavior of transformed cells. Through the use of Ad2- (non-oncogenic) and SV40- (highly oncogenic) transformed hamster cells, we have identified the phenotypic characteristics of these cells (e.g., Ad2 sensitivity and SV40 resistance to <u>in vitro</u> lysis by non-specific immune effector cells) that correlate with their ability to form tumors in immunocompetent hamsters. We also find that while Ad2- and SV40-transformed cells are equally active in production of transforming growth factors, only SV40-transformed cells secrete a powerful anti-mitogen, which may inhibit host defenses <u>in vivo</u> . We are also using SV40 to study the genetic basis of viral tissue tropism. We find that subcutaneously injected small t-antigen mutants of SV40 often induce abdominal B-cell lymphomas in hamsters, rather than the subcutaneous fibrosarcomas induced by wild-type SV40. The mutants may fail to produce a growth factor required for the <u>in vivo</u> transformation of non-proliferating cells.  In another project, we are studying the mechanism of <u>mutagenesis</u> , using an SV40-based shuttle vector as a probe to investigate the molecular mechanisms by which agents that damage DNA induce mutations in mammalian cells. Our studies on replication of UV-damaged SV40 DNA <u>in vivo</u> have led to a well-defined model of how the mammalian cell replication machinery responds to DNA damage, and at what steps in the replication process mutations become irreversibly established. Through use of the shuttle vector, we have extensively characterized the types of mutations that occur in mammalian cells either spontaneously or in response to DNA damage. Analysis of the <u>sequence specificity</u> of these mutations has led to a model which explains how the mammalian DNA polymerase introduces errors during DNA synthesis, causing mutations. Further studies with the vector in an <u>in vitro</u> DNA replication system should allow a correlation with the <u>in vivo</u> replication defects we observed.		

## ANNUAL REPORT

October 1, 1985 through September 30, 1986

### Epidemiology and Biometry Research Program, NICHD

The Epidemiology and Biometry Research Program (EBRP) conducts a broad range of epidemiological and statistical studies and clinical trials in the area of maternal and child health, pregnancy complications and birth outcome including low birthweight, preterm delivery and infant mortality. Other projects focus on Sudden Infant Death Syndrome and also on congenital malformations especially neural tube defects. Also studies are underway addressing infant feeding practices and their determinants as these relate to child health and development both in this country and abroad.

Clinical trials include a collaborative multi-center study of vaginal and cervical infection and its effect on the risk of low birthweight or preterm delivery and the management of cystinosis. EBRP is also actively involved in the development of clinical trials by the Maternal Fetal Medicine Network to address important issues of obstetrical management and by the Neonatal Intensive Care Network for the prevention and treatment of complications in the high risk neonate.

Several of the projects involve international activities. The Bedouin Infant Feeding Project in collaboration with Ben Gurion University in Beer Sheva, Israel has completed its data collection and the data are currently being analyzed. Another study in conjunction with Ben Gurion University focuses on maternal nutritional factors during pregnancy and their relationship to success and failure of breast feeding among a group of Jewish women of North African descent. A pregnancy outcome study is continuing in Shanghai County coordinated by Dr. Cai of Shanghai Medical University. This study is of considerable interest because of the reportedly very low rate of low birthweight in this area of China and will develop data on a population basis including risk factors. Data from this study should be available by the end of this year. A study of international comparisons of perinatal mortality involving Japan, Norway, Scotland, Western Australia and several states in the U.S.A. is underway.

In collaboration with the Aga Khan Medical University in Karachi, Pakistan a research project of risk factors associated with low birth weight and preterm deliveries in urban and rural settings of Pakistan is under development, which will also measure the impact of low birthweight and preterm delivery on child mortality and morbidity during the first two years of life. A study of inter-generational effects on birth outcome is planned with investigators from the Medical College of Vellore in India based upon a cohort study conducted from 1969 to 1974. Also a pilot study is underway in collaboration with the Department of Pediatrics, Krakow University, Poland to study risk factors associated with low birthweight in an area in Southern Poland known to have an unusual high rate of low birthweight.

Dr. Lechiam Naggan, Professor of Epidemiology Ben Gurion University in Beer Sheva, Isreal and Ms. Gillian Hundt, from the University of Warwick, United Kingdom have spent several weeks in Bethesda to work with EBRP on the analysis of the data from the Bedouin Infant Feeding study.

The EBRP is actively participating in the Assistant Secretary's task force on the Prevention of Low Birthweight which involves close collaboration with the Centers for Disease Control, the Division of Maternal and Child Health and the National Center for Health Statistics.

The EBRP is actively working with an outside group to develop a second workshop of major ongoing clinical trials to address the prevention of low birthweight or preterm delivery as a follow-up to the Evian Conference of May, 1985. This conference is tentatively planned for May or June 1987.

Dr. Liu Qi-pei, Professor of Nutrition from Shanghai Medical University has been a Visiting Fellow with the Epidemiology and Biometry Research Program during the past year and will return to his country at the end of July, 1986.

The EBRP has initiated a bi-monthly seminar series for and by its staff but also involving eminent investigators from this country and abroad. Outside speakers during the past year included Dr. John Fox, Professor, Social Statistics, Research Unit, City University, London, Dr. Carol Hogue, Chief, Pregnancy Epidemiology Branch, Centers for Disease Control, Dr. Michael Kramer, Associate Professor of Epidemiology and Pediatrics, University of Montrael in Canada, Dr. Emile Papiernik, Professor of Obstetrics and Gynecology, University of Paris, France, Dr. John Philips, Professor of Obstetrics, Rigshospitalet, Copenhagen, Denmark, Dr. Jack Pietrzyk, Professor of Pediatrics, University of Krakow in Poland, Dr. Jose Villar, Director, Nutrition Research of INCAP, Guatemala and Dr. Paul Wise, Assistant Professor, Harvard University.

#### Presentations:

Heinz W. Berendes, Factors Associated With Low Birthweight and What Can We Do To Prevent It? Prevention Research Coordinating Committee, National Institutes of Health, October 8, 1985, Bethesda, Maryland.

Heinz W. Berendes, Perinatal Factors Associated with Choice of Infant Feeding at Birth, American Public Health Association meeting, November 21, 1985, Washington, D.C.

Heinz W. Berendes, Chairperson, Low Birthweight in Developing Countries, American Public Health Association meeting, November 22, 1985, Washington, D.C.

Heinz W. Berendes, Intergenerational Effects on Low Birthweight, Perinatal Research Emphasis Centers meeting, May 15, 1986, Cincinnati.



# BIOMETRY BRANCH

- Z01 HD 00801-11     Studies Based on the Medical Birth Registries of  
Norway and Sweden  
H. J. Hoffman
- Z01 HD 00802-11     Studies of Linked Live Births-Infant Deaths and  
Fetal Deaths from U.S. States  
H. J. Hoffman
- Z01 HD 00803-02     Analysis of Sudden Infant Death Syndrome (SIDS)  
Risk Factors  
H. J. Hoffman
- Z01 HD 00811-07     National Collaborative Cysteamine Study Data Center  
G. F. Reed
- Z01 HD 00813-05     Biostatistical Methods for Laboratory Research Studies  
G. F. Reed
- Z01 HD 00818-05     Research in Developing Nonparametric Methods for  
Biomedical Applications  
G. F. Reed
- Z01 HD 00820-05     Statistical Methods for Epidemiologic Data  
D. W. Denman
- Z01 HD 00821-04     Development of New Graphical Methods for the Analysis  
of Biomedical Data  
D. W. Denman
- Z01 HD 00840-05     Statistical Discriminant Methods with Applications  
to Alcoholism Screening  
B. I. Graubard
- Z01 HD 00841-05     Methods for Comparing and Analyzing Data from  
Several Complex Surveys  
B. I. Graubard
- Z01 HD 00842-04     Development of Statistical Methods to Analyze  
Cluster Samples  
B. I. Graubard
- Z01 HD 00843-03     An Investigation of Matched Analysis in Case-  
Control and Cohort Studies  
B. I. Graubard
- Z01 HD 00844-03     Analysis of NHANES Anthropometric Measurements  
on Children  
B. I. Graubard



- Z01 HD 00850-10 Randomized, Controlled Study of Phototherapy for  
Neonatal Hyperbilirubinemia  
D. A. Bryla
- Z01 HD 00852-04 1980 National Natality Survey and Fetal Mortality Survey  
D. A. Bryla
- Z01 HD 00853-02 Design and Analysis of a Clinical Trial of Vi Poly-  
saccharide Vaccine  
D. A. Bryla
- Z01 HD 00854-02 Analysis of MCH Data from the National Logitudinal  
Youth Survey  
D. A. Bryla
- Z01 HD 00860-06 Analysis of Biomedical Time Series Data  
H. J. Hoffman
- Z01 HD 00861-04 Assessment of In-Utero Fetal Growth Patterns in  
Relation to Outcome at Birth  
H. J. Hoffman
- Z01 HD 00870-03 Long-Term Reproductive Effects of Cesarean Section Birth  
B. I. Graubard
- Z01 HD 00871-01 Clinical Trial of New Drug Therapy for Cystinosis  
G. F. Reed
- Z01 HD 00872-01 Factors Associated with Premature Births:  
Missouri Follow-back Survey  
D. A. Bryla





NICHD ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Biometry Branch

The Biometry Branch research activities are structured along three lines: (1) provision of statistical analysis and consultation to NICHD Intramural and Extramural investigators; (2) pursuit of individual and collaborative research in biometry, including both mathematical and biostatistical theory and applications; and (3) support of clinical trials initiated by the NICHD. The Branch maintains strong ties to both the Intramural and Extramural research programs of the Institute. Also, the Branch has supported a number of cooperative studies, including projects supported solely by NICHD and those receiving joint funding from other agencies within the U.S. Public Health Service.

The following review of Biometry Branch research activities is organized by subject matter, rather than by the statistical or mathematical methods utilized in the planning, design, conduct, or analysis phases of these research efforts.

Perinatal Morbidity and Mortality

Perinatal morbidity and mortality are key outcome variables for several studies being performed by the Biometry Branch. A major effort has been devoted to studies comparing United States data with that of two population-based perinatal data sets from Scandinavia, the Medical Birth Registries of Norway and Sweden.

A recently completed study using population-based data from the Norwegian Medical Birth Registry, 1967-76, has documented the strong tendency for mothers to repeat small-for-gestational age (SGA) deliveries in successive births. Mothers who showed this tendency ("repeater mothers") differed from mothers who had only one SGA delivery in their first three single births. In the group of mothers with only one SGA birth, there was an association between the SGA birth and such pregnancy complications as preeclampsia, vaginal bleeding, and placental pathologies. No similar association with medical complications during pregnancy was found for the repeater mothers. Instead, these mothers were characterized by lower educational attainment and lower socio-economic status based on husbands' occupational groupings. Thus, the tendency to repeat SGA birth appears to be mediated in part through more adverse living conditions and lifestyle habits.

In a follow-up study based on the Norwegian Medical Birth Registry, 1975-83, a similarly strong tendency for mothers to repeat SGA births in successive outcomes during the later time period has also been documented. The high relative risks shown in the first ten years of the registry were unchanged in terms of repeating SGA births during the later time period. The attributable risk for an SGA third birth, given that one or two of the previous births were SGA, was also high: accounting for approximately 50% of all such SGA third births. Moreover, if two previous SGA births had occurred then nearly half of

the subsequent births to these mothers were SGA. If, instead of SGA birth (<10th percentile of birth weight-for-gestational age), we substitute "very" SGA birth (<5th percentile) as the criterion, then the tendency to repeat this condition has even higher relative risks. At the other extreme of birth weight outcomes, the tendency to repeat large-for-gestational age (LGA) births (>90th percentile) or "very" LGA births (>95th percentile) shows similar high relative risks. Thus, these studies have documented the strong tendency for mothers to repeat similar outcomes in terms of birth weight and gestational age in successive pregnancies. One of the most notable results in the comparison between the two time periods was the lack of an appreciable fall in the perinatal mortality rate (PMR) in relation to SGA births. Overall, the PMR for sibships of three singleton births with one or more SGA births only dropped from 67.8 per 1,000 births in the earlier time period to 61.1 per 1000 births in the later time period. In contrast, the overall change in PMR in Norway between circa 1970 and circa 1980 was a 50 percent reduction in PMR from approximately 20 per 1,000 births to approximately 10 per 1,000 births.

Another recently completed study based on data from the Swedish Medical Birth Registry, 1976-80, compared intra-partum fetal mortality plus early neonatal mortality at different levels of medical care. Confounders were identified and adjusted for. The results of this study suggest that low birth weight infants have increased risks for intra-partum and early neonatal death if they are delivered at less specialized institutions compared to more specialized, but no such increased risks were found for infants with birth weights of 2500 grams or more. At the intermediate level (central county hospitals) the risks were moderately increased. The central county hospitals care for more than 50 percent of the deliveries in Sweden and their performance should be reassessed using material from more recent years because efforts have since been made to develop their competence in obstetrical surveillance and neonatal medicine. Subsequent studies relating perinatal outcome to the level of medical care available before, during and after birth will be carried out as part of the multinational study to compare birth weight-specific perinatal mortality rates.

In general, perinatal mortality rates provide a better indication of the availability, utilization, and effectiveness of health care for the pregnant woman and her fetus than the more traditional index of infant mortality. A recent publication based on data from four Nordic countries, 1900-1980, showed that "infant" mortality is a sensitive indicator of changing socio-economic circumstances, but perinatal mortality rates are more responsive to changes in underlying demographic factors--maternal age, parity and spacing between births--and to changes in the prenatal, obstetric, and pediatric care provided. A study currently underway in conjunction with the Office of International Statistics, National Center for Health Statistics is examining recent trends in perinatal and infant mortality rates over the past decade for the six countries participating in the International Collaborative Effort on Perinatal and Infant Mortality (ICE). The birth weight-specific comparisons used in this study provide documentation for the probable impact of such technological developments as neonatal intensive care units since 1970. Also, for international comparison of birth weight-specific perinatal mortality rates, we have shown that it is necessary to adjust for any differences in underlying birth weight distributions between populations in different countries before inferences can be made. However, for comparison through time within a country, birth weight distributions are sufficiently stable to make



direct comparisons of birth weight-specific perinatal mortality rates. One research effort that has emerged out of this general interest in perinatal morbidity and mortality is a prospective study designed to delineate risk factors for fetal growth retardation. Retarded fetal growth, defined as a birth whose weight is below the 10th percentile of birth weight-for-gestational age, is associated with increased rates of both perinatal mortality and morbidity. Using the research contract mechanism, this prospective study is being conducted at two locations: the University of Alabama in Birmingham and the University of Trondheim, Norway. The latter project also includes subcontracts with the University of Bergen, Norway and University of Uppsala, Sweden to supply additional data based on pregnancies and deliveries in these areas.

The aim of this research project is to determine risk factors which will distinguish mothers who have repeated small-for-gestational age (SGA) births from those mothers who have a single, unexpected SGA birth. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally via diagnostic ultrasound measurements and at delivery with standardized measurements. The study protocol includes recruitment of pregnant women before 17 weeks gestation and subsequent enrollment of women with high risk pregnancies through 33 weeks of gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy.

Pregnant mothers have been enrolled in this study over the past nine months, with several deliveries having already occurred. The plan is to continue enrolling pregnant mothers for one more year. At that time, we estimate that approximately 300 SGA births will have occurred in both the Alabama and Scandinavian sites. Study infants will be followed-up throughout the first year of life to assess catch-up growth, to monitor breast or bottle feeding patterns and occurrence of illnesses, and to assess the achievement of developmental milestones.

In another study, a secondary analysis of previously unpublished data obtained from the National Center for Health Statistics has been undertaken to review changes in perinatal and infant mortality by race in selected U.S. cities. Data from the current NICHD/NIEHS Study of Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates will also be used in assessment of recent time trends. This latter study is being carried out through the contract mechanism with the Departments of Health of five U.S. States --Michigan, Missouri, New York (Upstate), North Carolina, and Utah--and in four foreign countries--Australia (three States), Japan (Osaka province), Norway and Scotland. A uniform data tape format has been developed for the years 1980-84 and each contractor is preparing their data according to this format. A uniform procedure for classifying the "level" of medical care at birth has been achieved for the five U.S. States. The foreign participants will also define "level" of medical care in three broad categories (I, II or III). One of the principal aims of this study will be to compare the perinatal mortality attributable to "preterm" low birth weight infants in contrast to "SGA" low birth weight infants in the U.S. and each of the foreign countries. Standards defining the 10th percentile of birth weight-for-gestational age in each of the relevant population groups or subgroups are being developed. Each data set will contain some descriptors of education or occupation of parents so that some social factors can be controlled for.

Detailed cause-of-death (ICD-8 or ICD-9) information will also be available for study.

Perinatal mortality and morbidity data are also being examined in several other statistically diverse projects. Data from a variety of sources are being analyzed in different ways to study birth outcomes such as birth size, prematurity, and mortality. In collaboration with a former Visiting Scientist from Sweden, a matched case/control study has demonstrated that there is no adverse effect of a previous induced abortion on gestation or birth weight in the subsequent pregnancy, unless there had been a medical complication with the abortion. Another study has compared the outcome of deliveries of women who conceived with an IUD in place to those without an IUD present using a data set derived from the Kaiser Permanente Birth Defects Study. Also, in collaboration with Norwegian scientists, a data base of extensive longitudinal antenatal measurements, for example, symphysis-fundal heights, ultrasound measurements, hemoglobin, maternal weight-gain, and smoking, have been related to weight and length at birth in a Norwegian cohort. These results have produced information which may be useful clinically in assessing high risk pregnancies. In another research study conducted jointly with a Visiting Scientist from Norway, "precise" gestational age values have been determined for various subgroups within a large Scandinavian population of births.

The Biometry Branch is also working on research studies based on the 1980 National Natality Survey and 1980 National Fetal Mortality Survey conducted by the National Center for Health Statistics. The available data base is comprised of 9,941 live births and 6,386 fetal deaths. Initial maternal blood pressure readings during pregnancy have been analyzed in relation to a number of variables including birth outcome, maternal race, education, and age. Because of the small number of very low birth weight (VLBW) infants, <1500 grams, included in the 1980 National Natality Survey, we have undertaken a new research contract study to be conducted in 1987 in Missouri. Information will be obtained through mailed questionnaires to study mothers including all mothers of VLBW infants, all mothers of fetal deaths, and a sample of mothers with low birth weight infants (between 1500-2499 grams), and a sample of mothers with normal birth weight infants (>2500 grams). Data will also be obtained from vital records and medical records abstraction. Study infants will be assessed with a developmental screening test at one year of age.

#### Phototherapy Treatment for Neonatal Hyperbilirubinemia

Since 1974 the Biometry Branch has actively participated in the conduct of this clinical trial. This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing treated with untreated infants under specific conditions. The Biometry Branch has served as the data center for this study, and was the focal point for receipt of 1,339 newborn examinations and approximately 1,000 follow-up examinations per year until the children were six years of age. These forms were checked for accuracy, and precoded at each data collection center. The master files for each year's follow-up have been edited for keypunch and coding errors, and for internal consistency. The Branch has also been responsible for the analysis of the data.

During this year intensive effort has been exerted by a special working group to review the records of all the neurologically suspicious and abnormal cases.



The ultimate purpose of this review is to determine if there are any significant differences between the children treated with phototherapy and those that did not receive this treatment. If there are any significant differences the data will be analyzed to determine if bilirubin level and/or treatment modality is related. As part of the neurological examination timed movements, such as heel/toe, hand pats, were tested. Presently this data is being analyzed to see if there are any differences by sex, race or birth weight. Prior publications on timed movements have always been done on normal children. This analysis, it is hoped, will demonstrate if low birth weight results in any differences from the previous reports.

In 1985 two articles, one published and the other in press, discuss the effect of bright light in the nursery on the incidence of retinopathy of prematurity. As part of the Phototherapy Study, measurements of the intensity of the optical radiation (radiometric data) were obtained at each location. This information will be compared with the visual acuity of the less than 2,000 gram birth weight children to see if our large sample can offer any clues to visual development and retinopathy of prematurity.

#### Sudden Infant Death Syndrome (SIDS) Risk Factors

A major effort of the Branch has been invested in support of the NICHD Cooperative Epidemiological Study of the Sudden Infant Death Syndrome (SIDS) Risk Factors. All of the data collected for this study have been edited and entered onto computer files by staff of the Department of Biostatistics, School of Public Health and Community Medicine, University of Washington, Seattle. With final results of the pathology second review now available, we can state that SIDS was the final classification for 94.6% of the singleton, non turn-around infants submitted to the Pathology Coordinating Laboratory as having died of SIDS by the local medical examiners or coroners. Another 2.3% were classified as "possible" SIDS cases, while 1.4% were impossible to determine due to missing materials or vital information. Only 1.8% of the eligible SIDS infants were determined by the Pathology Review Panel to have died of "known" causes and were, therefore, non SIDS.

Based on the analysis of the 757 pathologically-defined singleton SIDS cases and both sets of matched, living controls (1,514 singleton living infants), the following descriptive information has emerged. Overall, 54 percent of the SIDS cases were black, although only 26 percent of the births in the study centers were black. Slightly less than 5 percent of SIDS cases were multiple births, but this represents an increased risk of two and one-half times greater than that for singleton births. The percentage of low birth weight infants is increased among cases by almost four-fold over random control infants (Controls A's). As expected because of matching, the percentage of low birth weight Control B infants is nearly identical to that of cases. The comparison between black and non-black infants revealed substantially higher rates of low birth weight among blacks, both for cases and Control A's.

Preliminary data on births and infant deaths in 1979 were provided by the National Center for Health Statistics so that the total incidence in our study areas could be compared with national figures. The national data agree quite well with the non-black SIDS incidence figures for the NICHD SIDS Cooperative Study at 1.2 per 1,000 live births. However, marked variations did occur between the different study centers. Moreover, the SIDS incidence among



blacks in the NICHD SIDS Cooperative Study was 35 percent higher than indicated by the national data. Further examination of infant and post-neonatal mortality rates by race for the NICHD SIDS Cooperative Study and the national data did not show any substantial differences. Thus, it may well be that SIDS is underreported as a cause of post-neonatal mortality, particularly among black infants in the United States.

During the last few years, some reports have suggested the possibility of a cause and effect relationship between immunization with diphtheria-tetanus-pertussis (DTP) vaccine and sudden infant death. The NICHD SIDS Cooperative Study collected data relevant to this issue both from maternal interviews and from the abstraction of medical records of the study infants. There were no differences found regarding a temporal association between DTP immunization and time of death for SIDS cases and time of interview for control infants. However, significantly fewer cases were ever immunized with DTP (39.8%) as compared to Control B, or Control A, infants (53.2% and 55.0%). This difference may reflect the fact that SIDS parents as a group generally did not have the same access to the medical care system. For example, significantly more SIDS cases (26.1%) received no regular well baby care compared to either set of age-matched control infants (15.3% and 15.6%).

In addition to the results quoted above, a number of expected findings have been confirmed by the NICHD SIDS Cooperative Study:

1. 18.2% of SIDS cases had gestation of less than 37 weeks compared to 4.6% of Control A's;
2. 59.7% of SIDS cases were male infants;
3. 31.5% of SIDS cases were born to women less than 20 years of age as compared to 17.2% for Control A and 21.7% for Control B infants;
4. only 26.6% of SIDS cases were first-born compared to 39.2% and 41.4% of Control A and B infants;
5. maternal smoking during pregnancy was reported in 69.6% of SIDS cases and in only 37.9% and 42.0% of Control A and B infants.

Also, the failure to find associations with a number of maternal variables which were previously suggested in the literature is of interest. Thus, there were no differences found between SIDS case and Control B mothers in the incidence of urinary tract infection, vaginitis, venereal disease or other maternal problems. No significant differences were found in C-section rates, use of maternal anesthesia and/or analgesia, or in the length of stages 1 and 2 of labor. There were no differences in the incidence of delivery complications, placenta previa, or in mean 1 and 5 minute Apgar scores. However, when compared to Control A infants, SIDS infants did have an increase in a number of nonspecific symptoms, including: respiratory distress, tachypnea, apnea of the newborn, tachycardia, cyanosis, pallor, irritability, poor feeding, jaundice, vomiting, abnormal cry, lethargy and tremors. After comparison to the ethnic and birth weight matched control, only tachypnea, tachycardia, cyanosis, and vomiting remained significant.

A special analysis was performed in regard to apnea, prematurity and growth retardation for presentation to an NIH Consensus Development Conference. The results demonstrated that almost all "apnea" in the newborn nursery occurred among preterm infants and there were no differences in the observed rate between SIDS infants and the birth weight- and race-matched control infants

(4%). The evidence from the study indicates that more SIDS cases experienced prenatal and post-natal growth retardation compared to control infants. Also, there were more documented post-neonatal episodes of "turned blue or stopped breathing" compared to either group of control infants (7% vs. 3%). These post-neonatal "apneic" episodes were reported by mothers at the time of interview. Only a very small number of these episodes led to emergency room visits or other medical attention. Also, 60% of these reported episodes were noticed only one time (the remainder were reported to have occurred two or more times). These recurrence rates did not differ between case and control infants.

### Diabetes in Early Pregnancy Study

Data collection for the Diabetes in Early Pregnancy (DIEP) Study ended this year, so that activity was concentrated on editing of the database and on plans for analysis. The first three topics of analysis will be pregnancy hormones, congenital malformations, and fetal loss.

Four hormones associated with pregnancy, beta human chorionic gonadotropin, glycoprotein hormone alpha subchain, human placental lactogen, and Schwangerschaftsprotein 1, were assayed from each study participant at weeks 6, 8, 10, 12, 20, 28, and 36 of gestation. The resulting profiles of hormone changes for each individual will be subjected to techniques such as analysis of variance for repeated measures, multivariate analysis of variance, and growth curve analysis to determine differences between diabetic and control women, differences due to variation in diabetic control among diabetic women, and differences in placental weight attributable to differences in the hormone profile.

The DIEP Study was designed in part to address the question of how diabetic control is related to the incidence in congenital abnormalities. Hypotheses to be tested are that good control has the same risk of malformations in diabetic women as in control women, and that among diabetic women those with better control experience a lower rate of malformations than the others. Crucial to this analysis is a definition of diabetic control that makes use of multiple daily measurements of indicators of hypoglycemia taken throughout gestation for each woman. Probable statistical approaches to this problem are control chart techniques that signal excursions from good control and discriminant analysis, which could identify linear combinations of the various measures that best distinguish the pregnancies with malformation outcomes from those without.

The third area of interest is the incidence of fetal loss, which can be addressed by the same analytic approaches as for congenital malformations, including adjustment by logistic regression, for covariate risk factors.

### Childhood Diseases or Disabilities

A major commitment of time and attention of the Biometry Branch is the use of the randomized clinical trial and its surrogates in order to advance research goals of the Institute. Concentrated in the Branch is expertise in the design, conduct, and analysis of comparative clinical studies that seek to evaluate the efficacy of therapeutic and preventive interventions. The Branch

is nearly always called upon to participate when some group in the Institute contemplates such a study.

An example of one of our efforts in the area is the long-standing commitment to evaluating therapies for nephropathic cystinosis, a rare inborn metabolic disorder characterized by a surfeit of cystine in the body's tissues that interferes with normal body growth and especially attacks the function of the kidneys. Renal dysfunction is progressive and culminates in end stage renal disease usually by age 10-15 years. Known to deplete the cystine content of human leucocytes in vivo, cysteamine was regarded as a candidate drug for retarding or stopping the deterioration of renal function due to cystinosis. This study was directed by a Principal Investigator at the University of California at San Diego and was organized to recruit cystinosis patients nationwide and provide the protocol and drug for their treatment. Data management and analysis was the responsibility of the the Biometry Branch with assistance from the Computer Science Section. Since the beginning of recruitment in 1978, 98 patients entered the study to receive 4 times daily oral doses of the drug for an average length of treatment of 33.4 months. Final evaluation was made this year of cysteamine as therapy for nephropathic cystinosis. The kidney function parameters, level of serum creatinine and creatinine clearance, were the outcome measures used for evaluation. For patients starting treatment with a serum creatinine level no greater than 2 mg/dl and receiving at least one year of treatment, mean end of study creatinine levels were 0.95 for cysteamine group and 1.41 for the control group. Creatinine clearance means were 43.8 ml/min/1.73 m<sup>2</sup> and 27.8 ml/min/1.73 m<sup>2</sup> for the two groups, respectively. Both sets of differences were significant at the .01 level and persisted after adjustment for baseline age, baseline serum creatinine, and age at end of study. These results support the claim that cysteamine can at least retard the course of renal dysfunction. An additional salutary effect of cysteamine is that body growth for those receiving cysteamine was closer to normal than that of the controls.

Although cysteamine has been judged to have a beneficial effect for victims of nephropathic cystinosis, many patients in the National Collaborative Cysteamine Study found the drug's unpleasant smell and taste so repugnant that they were unable to accept the full protocol dosage and therefore never received the full potential effect of cysteamine. The existence of another cystine-depleting agent, phosphocysteamine, which is more palatable than cysteamine, but is yet untested as a therapeutic drug, led to the establishment of a new clinical trial this year to evaluate alternatives to cysteamine. The University of California at San Diego has been contracted to conduct a randomized clinical trial to evaluate the effectiveness of phosphocysteamine relative to cysteamine on at least 80 patients to be enrolled in a 3-4 year period. Should some additional drug become available for evaluation within the coming year, such as a resin-based time release oral form of cysteamine, then it, too, may be included as a test drug.

Unlike the Cysteamine Study the new trial will have concurrent controls receiving cysteamine as the standard therapy. Prestratification based on baseline serum creatinine and previous cysteamine use will balance the assignment of treatment groups across prognostic factors. As in the Cysteamine Study analysis, the important outcome variables will be indicators of renal and glomerular function: serum creatinine and creatinine clearance. Data management and analysis is subcontracted to the University of Texas



Health Science Center at Dallas. Current study activities include protocol and forms development, testing of laboratory equipment and procedures, and publicity to enhance patient recruitment.

In another study, Branch staff are participating in the evaluation of the long-term effects to children exposed in infancy to chloride-deficient formula. In addition, staff have provided statistical support for the analysis of a small study consisting of about twenty children, who after being exposed to chloride-deficient formula (Neo-Mull-Soy), were evaluated at NIH when they were about two years old and re-evaluated two years later. Another study conducted in Sarasota, Florida compares school aged children who were exposed to chloride-deficient formula with those who ingested other soy-based formulas. In collaboration with other NICHD staff, the Branch is analyzing the cognitive scores collected from these children. One of the statistical questions that arose while designing these studies was what the gain would be by selecting a control from the same physician's practice (i.e., a neighborhood control) as the case. This has led to a research project to study empirically the validity and efficiency of neighborhood matching. A statistician in the Biomathematics Department of the University of California, Los Angeles is collaborating with Branch staff on this study.

Another area of collaborative research has been in the treatment, detection and risks of abusive drinking. Branch staff have continued to work with intramural researchers from the National Institute of Alcoholism and Alcohol Abuse (NIAAA) on the problems of finding biological markers for abusive drinking, and in characterizing patients who will be effectively treated for alcoholism. Statistical questions which have been addressed include the best way to derive a set of biological markers for detecting abusive consumption of alcohol and to select a discriminant function for the screening of heavy drinkers in a population. Research has been conducted into the robustness of quadratic, linear, and nonparametric discriminant functions and into potential benefits of applying simple rank and inverse normal score transformations to the original data. Related research efforts include the participation of Branch staff in collaboration with the Epidemiology Branch in studying the risk of malformed babies which are associated with prenatal consumption of alcohol.

The Biometry Branch in collaboration with the Laboratory of Developmental and Molecular Immunity, IRP, have trained field staff in Nepal for the Vi Polysaccharide Vaccine Trial. Staff have analyzed the data of the pilot studies (300 participants) for safety and immunogenicity. In March, staff participated in the full scale immunization. 3,500 participants received Vi vaccine and 3,500 a polyvalent pneumococcal vaccine in double blind format, using syringes filled at random and coded by the Institut Merieux. Immunization and six week reaction forms are being coded and keypunched for further study. This trial will last until March 1988 with visits made to participants every three days.

Another study, based on the 1981 Child Health Supplement, has included collaborative data development and analysis with the National Center for Health Statistics to produce reliable national descriptions of children's health. Two papers have been drafted for publication as journal articles on "The Health Status of Low Birth Weight Children in the U.S." and, also, "Complications of Childbirth: Self-Reporting from the Child Health

Supplement of the National Health Interview Survey Compared to Two Other Surveys." Future analysis plans include a more detailed analysis of the low birth weight children in terms of significant prenatal events and the childrens' later health outcome.

### Growth and Development

A significant amount of Branch staff effort has been in the nutrition and growth area. These efforts first began with the analysis of infant feeding data from the Pima Indian Reservation and the George Washington University Study, and have continued with the analysis of the Bedouin Arab Infant Feeding Study. The Pima Indian and the Bedouin Arab data sets were cluster samples including data on all the children in the family. The proper analysis of clustered data where binary observations within each cluster may be correlated is a statistical problem that has been investigated by Branch staff.

In addition, there has been collaboration with staff of the Epidemiology Branch involving several analyses of the first and second National Health and Nutrition Examination Surveys (NHANES I and II). In the process of analyzing the NHANES data it became clear that there were deficiencies in the statistical methodology for the analysis of complex survey data such as NHANES. This resulted in the development of a research contract to develop new methods for doing regression analysis on NHANES. A contract was awarded to the Research Triangle Institute in North Carolina to expand and develop regression methodology for complex surveys that can be applied to the analysis of growth and nutrition relationships in NHANES. This project, entitled Analysis of Relationships between Childhood Growth and Dietary Intake Using NHANES II, has just entered the second year. The work during the first year consisted of: (1) gathering potential relationships and hypotheses in the nutrition and growth areas that could be studied using the NHANES data sets; (2) working on the mathematical details relating to the estimation and evaluation of the parameters and test statistics for several different types of regression models; (3) obtaining sample design information from the Bureau of the Census and the National Center for Health Statistics that will be incorporated into the regression models; and (4) conducting preliminary analysis of lead intake and blood pressure from the NHANES II data set utilizing some of the newly developed regression models. It is still early to determine the practical value of the methods developed under this contract. However, the preliminary analysis showed interesting interrelationships between demographic sample design variables and blood lead level and blood pressure. During the second year of the contract, computer software will be developed for the new regression methods and used to further analyze the NHANES data.

Biometry Branch staff have also been involved with the Epidemiology Branch and Mental Retardation and Developmental Disabilities Branch, CRMC, in the planning and development of the Chorionic Villus Sampling and Amniocentesis Study. This multicenter clinical trial began its pilot phase in March, 1985. Preliminary analysis will begin soon on fetal loss rates and time to fetal death, with an appropriate application of life table techniques.

Several developmental studies utilizing statistical time series methodology have been performed by Branch staff. These applications have been shown to be valuable for the interpretation of a diverse collection of biomedical data

sets that were referred to the Branch for analysis. Digital filtering, spectral analysis, and new graphical display methods have been used to identify 20-60 second rhythms in human fetal heart rate recordings, 30-70 minute rhythms in the secretion of gonadotropins in male monkeys, and seasonal and weekly patterns in a 35-year record of oral temperature and pulse rate from one human subject. For example, in a paper published this past year, several encouraging findings were found in relation to the analysis of ultradian rhythms in human fetal heart rate. For example, it has been shown that standard techniques of statistical time series analysis can be usefully applied to conventional fetal heart rate recordings in order to investigate rhythms in fetal heart rate. Although evidence was found for a few specific ultradian rhythms in most of the 184 fetal heart rate recordings analyzed, it was not possible to further categorize them. In future studies, it is planned to examine ultradian rhythms in samples of normal and abnormal (complicated by diabetes or hypertension) pregnancies to determine whether these analyses could be of some clinical utility. In order to accommodate various applications of time series analysis techniques, special methods have been developed to accommodate short ( $n < 100$ ) as well as long ( $n > 10,000$ ) multivariate time series. Simulations and Monte Carlo methods have been used to evaluate the properties of these newly-devised techniques. The data findings as well as the statistical methodology have been reported in a variety of talks and papers, with several other studies currently underway.

#### Other Professional Activities

Mr. Denman continued to serve on the faculty as Adjunct Assistant Professor in the Department of Preventive Medicine and Biometrics of the Uniform Services University of the Health Sciences in Bethesda, Maryland.

Mr. Graubard has continued to work collaboratively on research projects with staff of the National Center for Health Statistics (NCHS). His expertise in the design and analysis of complex surveys has provided a beneficial link between our two agencies.

Mr. Hoffman has continued to serve as a member of the Planning Group for the International Collaborative Effort on Perinatal and Infant Mortality (ICE), a committee sponsored by NCHS and other U.S. Public Health Service agencies with additional members representing six other countries. The committee is chaired by Dr. Hartford, Office of International Statistics, NCHS.

At the request of the Scientific Review Program, NICHD, Dr. Reed and Mr. Hoffman provided statistical expertise for the analysis of priority scores for several of the Institute's review committees. The purpose of this statistical investigation is to assess the efficiency of the review process, and to identify possible means of improving the process, by providing reliable aggregate data to the Director, NICHD and members of these committees.

Mr. Hoffman has been invited to serve on the advisory committee for a new study investigating possible associations between pertussis vaccination and risk for either subsequent encephalopathy or the Sudden Infant Death Syndrome (SIDS) based on data obtained through the Boston Collaborative Drug Surveillance Program.



NICHD ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Biometry Branch

Publications:

Bakketeig, L.S., Bjerkedal, T., and Hoffman, H.J.: Small-for-gestational age births in successive pregnancy outcomes: Results from a longitudinal study of births in Norway. Early Human Devel. (In press).

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Meirik, O., Denman, D.W., Hoffman, H.J., Fetterly, K., and Villar, J.: Neonatal mortality and level of care in Sweden 1976-80. Lakartidningen 83: 691-694, 1986.

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Rawlings, R.R., Graubard, B.I., Faden, V.B., and Eckardt, M.J.: A study on discriminant analysis techniques applied to multivariate lognormal data. Journal of Statistical Computation and Simulation. (In press).

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Steardo, L., Marone, E., Barone, P., Denman, D.W., Moteleone, P., Cardone, G.: Prophylaxis of Migraine attacks with a  $Ca^{++}$  entry blocker: Flunarizine vs. Methysergide. Journal of Clinical Pharmacology. (In press).

van Belle, G., Hoffman, H.J., and Peterson, D.R.: Intrauterine growth retardation and the Sudden Infant Death Syndrome. In Harper, R.M., and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, Spectrum Scientific Publications. (In press).

## Presentations:

Denman, D.W., Reed, G.W., Hoffman, H.J., Jacobsen, G., and Bakketeig, L.: Least squares and empirical Bayes estimation of symphysis fundus growth curve related to birth weight. Contributed paper for the XIII International Biometric Conference. Seattle, Washington, July 1986.

Denman, D.W.: Introduction to SASGRAPH. Invited presentation at the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences. Bethesda, Maryland, January, 1986.

Hoffman, H.J., Denman, D.W., Dawson, A.J., and Dalton, K.J.: A computer analysis of short term ultradian rhythms in human fetal heart rate. Contributed paper for the XI World Congress of Gynecology and Obstetrics. Berlin (West), Federal Republic of Germany, September, 1985.

Hoffman, H.J., and Bergsjø, P.: Trends in birth weight-specific perinatal mortality: 1970-82. Invited presentation for a Symposium on Perinatal and Infant Mortality -- An International Perspective presented at the Annual Meeting of the American Public Health Association. Washington, DC, November, 1985.

Hoffman, H.J.: Apnea, Birth Weight, and SIDS. Invited presentation at the Public Panel Meeting of the Consensus Development Conference on Infantile Apnea and Home Monitoring held at the Lister Hill National Center, National Institutes of Health. Bethesda, Maryland, May, 1986.

Hoffman, H.J.: Methodological considerations for the collection of obstetric and demographic data for the 1988 National Maternal and Infant Health Survey (NMIHS). Invited presentation for the All Day Planning Conference for the 1988 NMIHS, Session on Obstetric Complications and Technology Issues, National Center for Health Statistics. Hyattsville, Maryland, May, 1986.

Hoffman, H.J., Denman, D.W., Haldorsen, T., and Bakketeig, L.S.: Birth weight and perinatal mortality: Impact of biological covariates. Invited presentation for the 1986 Joint Statistical Meetings of the American Statistical Association. Chicago, Illinois, August, 1986.

Hoffman, H.J., and Damus, K.H.: SIDS risk factors -- Results of the NICHD SIDS cooperative epidemiological study. Invited presentation for the 1986 Western Regional SIDS Conference. Oakland, California, September, 1986.

Graubard, I.: A study of discriminant analysis techniques applied to multivariate lognormal data. Contributed paper for the 1986 Joint Statistical meetings. Chicago, Illinois, August, 1986.

Graubard, I.: Neighborhood controls. Invited presentation for the Epidemiology Training Program, NIH. Bethesda, Maryland, June, 1986.

Jacobsen, G., Hoffman, H.J., Denman, D.W., Reed, G.W., Bakketeig, L.: Deviations in intrauterine growth patterns as detected by serial symphysis-fundal height measurements. Contributed paper for the X European Congress in Perinatal Medicine. Leipzig, German Democratic Republic, August, 1986.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00801-11 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies Based on the Medical Birth Registries of Norway and Sweden

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB EBRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB EBRP NICHD  
Ernest E. Harley Chief CS EBRP NICHD  
Heinz W. Berendes Director EBRP NICHD

COOPERATING UNITS (if any) Inst. of Hygiene and Social Medicine and Dept. of OB/GYN, Univ. of Bergen, Norway (P. Bergsjø and R. Skjaerven); Dept. of Community Medicine, Univ. of Trondheim and Nat'l Inst. of Public Health, Oslo, Norway (L. Bakketeig, T. Haldorsen); Dept. of Social Medicine, Uppsala Univ. (O. Meirik).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.4

## PROFESSIONAL:

.2

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway and Sweden, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, (3) perinatal mortality in relation to order of birth and size of sibship, (4) epidemiologic risk factors for preterm birth, and (5) epidemiologic risk factors for small-for-gestational age births.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00802-11 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB EBRP NICHD

Others: Heinz W. Berendes Director EBRP NICHD  
Anne Willoughby Epidemiologist EB EBRP NICHD  
Mary D. Overpeck Health Statistician EB EBRP NICHD  
Allen J. Wilcox Acting Chief EB BRAP NIEHS

## COOPERATING UNITS (if any)

Departments of Health in the following states: Michigan, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, National Center for Health Statistics (R. Hartford).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.4

## PROFESSIONAL

.2

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

Several research contracts have been jointly funded by NICHD and NIEHS to provide data from selected U.S. States (listed above) to compare with data from other developed countries (Australia, Japan, Norway and Scotland) for the time period, 1980-84. This study is entitled: Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00803-02 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Howard J. Hoffman Chief BB EBRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB EBRP NICHD  
Karla H. Damus Consultant BB EBRP NICHD  
Heinz W. Berendes Director EBRP NICHD  
Eileen G. Hasselmeyer Director SRP NICHD  
Jehu C. Hunter Consultant SRP NICHD

COOPERATING UNITS (if any) U. Washington (D. Peterson; G. van Belle); Loyola U. (J. Goldberg); U. Calif., Davis (J. Kraus); N.Y. Med. Health Res. Assoc. (J. Pakter); N.Y. State Health Dept. (D. Janerich); St. Louis MCH Council (L. Hillman); U. Calif, L.A. (R. Harper) and U. London, U.K. (D. Southall); AFIP, Washington, D.C. (T. Stocker).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.4

## PROFESSIONAL:

.3

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NICHD Cooperative SIDS Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 838 SIDS cases (800 singleton and 38 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of neuro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons will be made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00811-07 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) National Collaborative Cysteamine Study Data Center		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	George F. Reed	Mathematical Statistician BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician BB EBRP NICHD
	Ernest E. Harley	Chief CS EBRP NICHD
	Elva Nelson	Statistical Assistant CS EBRP NICHD
	William Gahl	Senior Staff Fellow HGB IRP NICHD
COOPERATING UNITS (if any) Univ. California, San Diego (J. Schneider); Uniform Services Univ. of the Health Sciences (J. Schlesselman); Univ. of Michigan Medical School (J. Thoene).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS: .4	PROFESSIONAL: .2	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided ) This study was a <u>clinical trial</u> to determine the <u>safety and efficacy</u> of <u>cysteamine</u> in the treatment of <u>nephropathic cystinosis</u> , a rare inborn <u>metabolic disease</u> which usually leads to <u>end-stage renal disease</u> before 10 years of age. All children enrolled in the trial received oral cysteamine. <u>Control</u> information was provided by data collected on 64 patients who participated <u>in a</u> previous trial evaluating <u>ascorbic acid</u> for the treatment of this disease. The cysteamine trial enrolled 94 <u>patients</u> ; analysis of data is completed, and publication of results is pending review.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00813-05 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biostatistical Methods for the Analysis of Laboratory Research Studies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	George F. Reed	Mathematical Statistician	BB	EBRP	NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB	EBRP	NICHD
	Barry Graubard	Mathematical Statistician	BB	EBRP	NICHD
	Howard J. Hoffman	Chief	BB	EBRP	NICHD
	Phil Skolnick	Chief	NS	LBC	NIADDK
	Ronald Elin	Chief		CPD	CC
	Mark Ruddell	Medical Technologist		CPD	CC
	David W. Alling	Special Assistant/Biometry		IRP	NIAID

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.2

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research in design and analysis problems arising from laboratory studies on:  
(1) dose-response relationships, (2) bioassay and potency estimation,  
(3) time to event, life table analyses, and (4) other investigations of the effects of external stimuli.

In addition to work on a technique for estimating tolerance limits for chemical residue depletion in animals, which has been submitted for publication, a major effort in this research area has arisen in the analysis of data from the Clinical Center's Normal Range Study. This study has resulted in the collection of a large number of biochemical and clinical measurements taken serially for 2 1/2 years from "normal" volunteers. The object of the analysis is to characterize the distribution of each variable in order to determine values that can be considered normal. Some of the statistical techniques to be applied will be exploratory data analysis methods, including graphical techniques and outlier detection, transformation of variables, analysis of variance components, and serial correlation. The results of this project will appear in several published reports of quantitative characterizations with special reference to factors that may affect these distributions, such as smoking, drinking, and eating habits, and other demographic or socio-economic factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00818-05 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders ) Research in Developing Nonparametric Methods for Biomedical Applications		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	George F. Reed	Mathematical Statistician BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician BB EBRP NICHD
	Howard J. Hoffman	Chief BB EBRP NICHD
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS .2	PROFESSIONAL: .2	OTHER: .0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided )  <p>The objective is to investigate and develop distribution-free methods in areas of application for which standard parametric techniques are <u>inappropriate</u> or <u>too sensitive</u> to violations of underlying assumptions.</p> <p>Much of the work of the Branch lends itself to the nonparametric approach. In sample size studies involving analysis of 2x2 tables, the determination of the <u>minimum detectable risk</u> for a given sample size is often required. Some <u>asymptotic techniques</u> have been developed in the Branch for this, but they must ultimately be validated by an exact technique which is theoretically based on the theory of <u>randomization testing</u>. This technique is now being developed. Another general application is the use of <u>runs tests</u> to evaluate <u>residuals in regression analysis</u> to determine goodness of <u>fit</u>. Research on a particularly apt nonparametric runs test, based on the variance of the length of positive and negative runs of residuals, continues. Investigation is also continuing in the use of randomization testing for comparing proportions with <u>cluster effects</u>.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00820-05 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methods for Epidemiologic Data

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel W. Denman III Mathematical Statistician BB EBRP NICHD

Others: Barry I. Graubard Mathematical Statistician BB EBRP NICHD

Howard J. Hoffman Chief BB EBRP NICHD

George F. Reed Mathematical Statistician BB EBRP NICHD

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.3

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since many epidemiologic problems cannot be solved by standard techniques, new methods can help extract more complete answers from research data. The objective of this project is to use mathematical theory and computer simulations to develop and evaluate statistical methods appropriate to data arising in epidemiologic research, and to carry out the statistical programming needed to make these methods easily available to other researchers. This may include evaluating outside computer software, using standard programs in novel ways, and writing special purpose programs.

Further study will continue in the use of generalized linear models and the SAS procedure GLM in regression, analysis of variance, and analysis of covariance. Special interest will be paid to the use of logistic regression, log-linear models, and covariate adjustment using empirical Bayes techniques. Useful techniques will be presented in seminars and publications in statistical journals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00821-04 BB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Development of New Graphical Methods for the Analysis of Biomedical Data

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Daniel W. Denman III Mathematical Statistician BB EBRP NICHD

Others: Howard J. Hoffman Chief BB EBRP NICHD  
George F. Reed Mathematical Statistician BB EBRP NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS

.2

PROFESSIONAL:

.2

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Statistical graphics are an integral part of the analysis and presentation of data. Rapid development in this field is evidenced by an extensive research literature and a host of new computer graphics technologies.

The object of this project is to draw from current literature and computer demonstrations and develop graphical methods for: (1) more effective statistical analysis, particularly of multi-dimensional data sets and time-dependent variables; and (2) for more easily understood summaries in finished presentations. This may include acquiring new computer hardware and software from outside sources, as well as making full use of support provided by DCRT and developing original methods using existing resources.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00840-05 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Statistical Discriminant Methods with Applications to Alcoholism Screening

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB EBRP NICHD

## COOPERATING UNITS (if any)

Alcohol, Drug Abuse and Mental Health Administration (R. Rawlings, S. Teper, V. Fadden and M.J. Eckardt).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.1

## PROFESSIONAL:

.1

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study investigates the statistical properties of a variety of discriminant functions and determines how well they differentiate between alcoholic, other diseased, and normal populations using standard batteries of blood chemistries. Blood chemistry variables that are used to discriminate between diseased and normal groups have been found to have skewed distributions. Using computer simulations, the properties of parametric (linear and quadratic) and nonparametric (fixed and variable kernel) discriminant methods have been investigated when the data comes from a skewed multivariate lognormal distribution. In addition, rank and inverse normal score transformations were applied to the data from the simulation in order to determine if they could improve upon the accuracy of the discriminant functions. It was found that the nonparametric methods were less accurate than the parametric methods when the data came from a multivariate lognormal distribution. The rank and inverse normal score transformations greatly improved the classification accuracy of the parametric methods. This work will aid researchers in developing medical screening procedures that are based upon statistical discriminant methods.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00841-05 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Methods for Comparing and Analyzing Data from Several Complex Surveys

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB EBRP NICHD

Others: Howard J. Hoffman Chief BB EBRP NICHD  
Dwight B. Brock Mathematical Statistician EDB NIA

## COOPERATING UNITS (if any)

Research Triangle Institute (B.V. Shah).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS

.2

## PROFESSIONAL:

.2

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This study will develop statistical methods for the analysis of data from complex designed surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). Existing multiple linear regression methods for the analysis of data from complex surveys are compared to newly developed regression methods. These regression methods will be applied to the NHANES data sets to determine if they can be used to provide new information on the complex relationships of growth and nutrition. The preliminary results from this research indicate that the newly developed regression models can better describe complex relationships in the data. This research is being pursued in part through a research contract with the Research Triangle Institute to work in collaboration with NICHD to carry out this study. Over the course of this contract, manuscripts will be prepared for publication which will present the results of the study along with the development of computer programs for applying the methods to real data.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00842-04 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Statistical Methods to Analyze Cluster Samples

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Barry I. Graubard	Mathematical Statistician	BB EBRP	NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP	NICHD
	Mitchell Gail	Chief	BB EBP	EMS NCI
	Thomas Fears	Mathematical Statistician	BB EBP	EMS NCI

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS.

.2

## PROFESSIONAL:

.2

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research project will study statistical methods for analyzing categorical data that comes from cluster samples where the observations within each cluster may be correlated and where the observations may be selected with unequal probabilities. In particular, the analysis of cluster samples from population-based case-control studies and cross-sectional and longitudinal health surveys is examined. Research has concentrated on developing modifications to logistic regression and Mantel-Haenzel and Wolf-Haldane procedures that would account for the complex sample design. Computer simulations are used to validate statistical approximations used in the development of modified methods. Preliminary results from this research indicate that the modified methods for analyzing data from cluster samples appropriately take into account the intra-cluster correlation structure and the unequal weighting of the observations. These methods will be useful for analyzing infant feeding studies and repeat pregnancy studies where the family constitutes the cluster.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00843-03 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) An Investigation of Matched Analysis in Case-Control and Cohort Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Barry I. Graubard	Mathematical Statistician BB EBRP NICHD
Others:	Howard J. Hoffman George F. Reed	Chief Mathematical Statistician BB EBRP NICHD BB EBRP NICHD
COOPERATING UNITS (if any) Biomathematics Department, School of Medicine, UCLA (E. Korn).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS: .2	PROFESSIONAL: .2	OTHER: .0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>             This study will investigate the validity and efficiency of neighborhood matching for case-control and cohort studies. The National Health and Examination Surveys I and II data were used in conjunction with neighborhood codes (i.e., specifying which individuals in the sample lived close together) to empirically determine the effect neighborhood matching would have upon validity and variance of estimates of risk of various conditions with respect to differing exposures. It was demonstrated that for some types of exposure-condition relationships, neighborhood matching was useful for controlling for confounding. However, there was a loss in efficiency due to a reduced number of matchable observations and a smaller number of degrees of freedom in the test statistics. These empirical examples can provide some guidance to researchers who contemplate neighborhood matching for an observational study. This project is one of the first known attempts of investigating the effect neighborhood matching has upon the analysis of observational data.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00844-03 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of NHANES Anthropometric Measurements on Children

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Barry I. Graubard Mathematical Statistician BB EBRP NICHD

Other: Natalie Kurinij Nutritionist EB EBRP NICHD

## COOPERATING UNITS (if any)

School of Public Health, Yale University (P. Rosenberg).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.05

## PROFESSIONAL:

.05

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will develop a new weight-for-height index which adjusts for the age of the child. Relative weight indices such as Quetelet and Ponderal indices that have been used on adult populations do not work well for children. They tend to be strongly correlated with age and are not predictive of body fat. Polynomial regression models that include age terms were fitted to weight and height data for children between the ages of 3 to 12 years from the National Health and Nutrition Examination Survey II in order to estimate a standardized weight-for-height which is adjusted for the age of the child. Separate regression models were estimated for boys and girls. A relative weight index was established using the estimated standard weights from the regressions. This relative weight index was compared to triceps and subscapular skinfold measurements. The new relative weight index had the desirable properties of being uncorrelated with age and highly associated with skinfold measurements. The derived relative weight index can be used in the analysis of epidemiologic studies that require an easily calculated index of obesity among prepuberty children.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00850-10 BB																																				
PERIOD COVERED October 1, 1985 to September 30, 1986																																						
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia																																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Dolores A. Bryla</td> <td style="width: 20%;">Statistician</td> <td style="width: 10%;">BB</td> <td style="width: 10%;">EBRP</td> <td style="width: 10%;">NICHD</td> </tr> <tr> <td>Others:</td> <td>Howard J. Hoffman</td> <td>Chief</td> <td>BB</td> <td>EBRP</td> <td>NICHD</td> </tr> <tr> <td></td> <td>Barry I. Graubard</td> <td>Mathematical Statistician</td> <td>BB</td> <td>EBRP</td> <td>NICHD</td> </tr> <tr> <td></td> <td>Heinz W. Berendes</td> <td>Director</td> <td></td> <td>EBRP</td> <td>NICHD</td> </tr> <tr> <td></td> <td>Peter Scheidt</td> <td>Medical Officer</td> <td>HLBB</td> <td>CRMC</td> <td>NICHD</td> </tr> <tr> <td></td> <td>Karin B. Nelson</td> <td>Chief</td> <td>CPMDS</td> <td>DNB</td> <td>CDNDP NINCDS</td> </tr> </table>			PI:	Dolores A. Bryla	Statistician	BB	EBRP	NICHD	Others:	Howard J. Hoffman	Chief	BB	EBRP	NICHD		Barry I. Graubard	Mathematical Statistician	BB	EBRP	NICHD		Heinz W. Berendes	Director		EBRP	NICHD		Peter Scheidt	Medical Officer	HLBB	CRMC	NICHD		Karin B. Nelson	Chief	CPMDS	DNB	CDNDP NINCDS
PI:	Dolores A. Bryla	Statistician	BB	EBRP	NICHD																																	
Others:	Howard J. Hoffman	Chief	BB	EBRP	NICHD																																	
	Barry I. Graubard	Mathematical Statistician	BB	EBRP	NICHD																																	
	Heinz W. Berendes	Director		EBRP	NICHD																																	
	Peter Scheidt	Medical Officer	HLBB	CRMC	NICHD																																	
	Karin B. Nelson	Chief	CPMDS	DNB	CDNDP NINCDS																																	
COOPERATING UNITS (if any) Downstate Medical Center, State Univ., N.Y.; Albert Einstein College of Medicine; Long Island Jewish-Hillside Medical Center; Medical College of Virginia; Univ. of Southern California Medical Center; Univ. of Cincinnati; Computing Sciences Consultant (K. Fetterly).																																						
LAB/BRANCH Biometry Branch																																						
SECTION																																						
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892																																						
TOTAL MAN-YEARS: .6	PROFESSIONAL: .4	OTHER: .2																																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             This study, which began in 1974, is a cooperative, randomized clinical trial to determine the <u>safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia</u> by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. <u>Physical, neurological and mental development</u> of these infants were followed through six years of age.           </p> <p>             The Biometry Branch served as a <u>data center</u> for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency. The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. The results of the newborn data were published in a supplement to Pediatrics in February 1985. It is anticipated that manuscripts on the <u>follow-up data</u> will be submitted for publication by the end of 1986.           </p>																																						

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00852-04 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1980 National Natality Survey and Fetal Mortality Survey

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB EBRP NICHD

Others: Howard J. Hoffman Chief BB EBRP NICHD  
Donald McNellis Medical Officer/Obstetrics PPB CRMC NICHD

## COOPERATING UNITS (if any)

National Center for Health Statistics, Division of Vital Statistics, Natality Statistics Branch (P. Placek).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.1

## PROFESSIONAL:

.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The 1980 National Natality Survey and 1980 National Fetal Mortality Survey conducted by the National Center of Health Statistics (NCHS) contains data on 9,941 live births and 6,386 fetal deaths. For each live birth and fetal death certificate selected, mother, physician, hospital and radiation questionnaires were obtained by NCHS. This project has provided data on a nationwide sample relating to pregnant women's characteristics, outcome of pregnancy, labor and delivery.

During this year, planning meetings for the proposed 1988 National Maternal and Infant Health Survey (NIMIHS) were held with the National Center of Health Statistics (NCHS). It is proposed that information will be collected for three national samples of vital records: 10,000 certificates of live births, 6,000 reports of fetal deaths, and 4,000 death certificates for infants. Based on the earlier collaboration with the Biometry Branch for the 1980 surveys, NCHS staff has worked closely with us in formulating the proposed content of questionnaires for NIMIHS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00853-02 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Dolores A. Bryla	Statistician BB EBRP NICHD
Others:	George F. Reed	Mathematical Statistician BB EBRP NICHD
	Charles V. Lowe	Special Assistant/Director OD NICHD
	John Robbins	Chief LBMI NICHD
COOPERATING UNITS (if any) TEKU Hospital, Nepal (I. Acharya); Uniformed Services University of the Health Sciences (J. Schlesselman).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>This study is a cooperative, <u>randomized trial</u> to determine the <u>efficacy</u> of <u>Vi polysaccharide</u> in preventing <u>typhoid fever</u> in Nepal. The Biometry Branch's <u>involvement</u> in this study is to <u>design data collection forms</u>, and assist in the <u>data management</u> and the analysis with the study investigators from NICHD and Nepal.</p> <p>In October, a <u>pilot study</u> was done to verify the <u>immunogenicity</u> of the vaccine. The analysis of this data was performed by the Biometry Branch. In March 1986, 6,912 volunteers from five villages in Nepal were randomly vaccinated with either the Vi polysaccharide or pneumococcal vaccine. These volunteers will be visited every three days for the next two years to verify their health status and to detect any typhoid cases prior to treatment. <u>Blood cultures</u> will be done on anyone with a fever of three days duration. The results of the randomization will not be available until late in 1988.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00854-02 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of MCH Data from the National Longitudinal Youth Survey

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dolores A. Bryla Statistician BB EBRP NICHD

Others: Howard J. Hoffman Chief BB EBRP NICHD  
Natalie Kurinij Nutritionist EB EBRP NICHD  
Donald McNellis Medical Officer/Obstetrics PPB CRMC NICHD

## COOPERATING UNITS (if any)

Ohio State University (F. Mott); R.W. Johnson Clinical Scholars Program,  
University of North Carolina (C. Homer).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.1

## PROFESSIONAL:

.1

## OTHER:

.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has as its primary objective to analyze and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. In addition, a wealth of other data have been collected on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The collection of data on pregnancy outcome and the first year of life of the child began in 1983. The data tape for 1985 should be available for use by the end of calendar year 1986. With this three year data base, analysis of trends over time in the maternal and child health can be done.

The Biometry Branch has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 HD 00860-06 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders ) Analysis of Biomedical Time Series Data		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Howard J. Hoffman      Chief	BB EBRP NICHD
Others:	Daniel W. Denman III      Mathematical Statistician Mary Ann Brock      Biologist	BB EBRP NICHD CI CP GRC NIA
COOPERATING UNITS (if any) Department of Pediatrics, University of South Florida College of Medicine, St. Petersburg, Florida (B. Bercu); Pediatric Nutrition, Mead Johnson Company (J. Hansen); Department of Obstetrics & Gynecology, University of Cambridge, England (K. Dalton).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS: .4	PROFESSIONAL: .2	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use stenderd unreduced type. Do not exceed the spece provided.)  The objectives of this project are: (1) to characterize developmental patterns from daily measurements of <u>gonadotropins</u> and for <u>estrogens</u> in <u>premenarchial girls</u> and <u>pubescent boys</u> based on <u>radioimmunoassay</u> methods for measuring <u>urinary luteinizing hormone</u> , <u>urinary follicle stimulating hormone</u> , and <u>urinary estradiol</u> , <u>estriol</u> and <u>estrone</u> hormones; (2) <u>gonadotropins</u> in both castrated and intact <u>male monkeys</u> of different ages; (3) <u>growth hormone</u> in <u>normal</u> and <u>precocious pubertal children</u> ; (4) to assess <u>circadian</u> and other <u>rhythms</u> in <u>heart rate</u> , <u>temperature</u> and other <u>serial data</u> collected from long-term studies in humans; and (5) to perform analysis of these serial measurements using methods of <u>statistical time series analysis</u> , including <u>autoregressive filtering</u> , <u>auto-</u> and <u>cross-spectrum analysis</u> , and <u>robust smoothing</u> procedures.		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00861-04 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Howard J. Hoffman	Chief	BB	EBRP	NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB	EBRP	NICHD
	Heinz W. Berendes	Director		EBRP	NICHD
	Donald McNellis	Medical Officer/Obstetrics	PPB	CRMC	NICHD

## COOPERATING UNITS (if any)

Dept. of Community Medicine, Univ. of Trondheim, Norway (G. Jacobsen and L. Bakketeig); Dept. OB/GYN, Univ. of Bergen, Norway (P. Bergsjø); Dept. OB/GYN, Uppsala Univ., Sweden (G. Lindmark); Bell Communications, Livingston, N.J. (G.W. Reed); Dept. OB/GYN, Univ. of Alabama in Birmingham (R. Goldenberg).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.4

## PROFESSIONAL:

.3

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unraduced type. Do not exceed the space provided.)

This project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala). The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00870-03 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Long-Term Reproductive Effects of Cesarean Section Birth		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Barry I. Graubard	Mathematical Statistician BB EBRP NICHD
Others:	Howard J. Hoffman Ntinios Myrianthopoulos	Chief Section Chief BB EBRP NICHD DNB CDNDP NINCDS
COOPERATING UNITS (if any) University of Helsinki, Department of Public Health, Finland (K.E. Hemminki); New York State Department of Health (D. Glebatis, D. Janerich and G. Therriault); National Center for Health Statistics, Family Growth Branch (W. Mosher).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS: .05	PROFESSIONAL: .05	OTHER: .0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The purpose of the work is to study long-term adverse effects possibly following a delivery with cesarean section. Effects on subsequent fertility, ectopic pregnancies and on malformations of subsequent children having been studied using U.S. data. Subsequent fertility is studied by comparing women having had a cesarean section to those having had a vaginal delivery in their first pregnancy using data from the 1982 National Survey of Family Growth. Effect on ectopic pregnancies is studied by comparing the past delivery history of women having had ectopic pregnancy to that of women having had a live birth or a spontaneous abortion. The data source is fetal and live birth certificates in Upstate New York. Effects on malformations are studied by comparing the malformation rates of children whose mothers have had a previous cesarean section to that of children whose mothers have had a previous vaginal delivery. The data source is the Collaborative Perinatal Project. Many different types of problems, both for the mother and infant, in the subsequent pregnancies have been studied using the data in the Swedish Birth Register. Subsequent studies include linking this data to the hospital discharge register to study problems not related to pregnancies ending in birth.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00871-01 BB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Trial of New Drug Therapy for Cystinosis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George F. Reed Mathematical Statistician BB EBRP NICHD

Others: Daniel W. Denman III Mathematical Statistician BB EBRP NICHD  
William Gahl Senior Staff Fellow HGB IRP NICHD

## COOPERATING UNITS (if any)

Univ. California, San Diego (J. Schneider); Univ. of Michigan Medical School (J. Thoene); Univ. of Texas Health Science Center, Dallas (J. Reisch).

## LAB/BRANCH

Biometry Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.3

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Cysteamine Study provided answers to the question of the drug's efficacy with some inferential difficulty, since cysteamine's unpleasant taste and smell rendered it unpalatable to many patients, who subsequently did not receive effective amounts of the drug. The design of the study itself, with no randomized concurrent control group, obscured effects and required a good deal of reliance on adjustment techniques in the final analysis.

There exists a chemical analog to cysteamine, phosphocysteamine, which is more palatable and demonstrates cystine depleting properties, although it has not been subjected to a rigorous clinical test of efficacy. The object of the study is to compare treatment with phosphocysteamine to cysteamine therapy in a randomized clinical trial. If some other drug with therapeutic promise is made available early enough in the study, then it, too, may be included in trial.

Patient recruitment and treatment will be coordinated at a contracted study center at the University of California, San Diego. Data center functions will be performed at the University of Texas Health Science Center at Dallas. The study will encompass 3-4 years of enrollment and treatment of at least 80 patients. The drug will be evaluated on the basis of renal function as measured by serum creatinine levels and creatinine clearance, as a surrogate of glomerular filtration rate, at the end of the study.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 HD 00872-01 BB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Factors Associated with Premature Births: Missouri Follow-back Survey		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Dolores A. Bryla	Statistician BB EBRP NICHD
Others:	Howard J. Hoffman Anne Willoughby	Chief Epidemiologist BB EBRP NICHD EB EBRP NICHD
COOPERATING UNITS (if any) Missouri Division of Health (G. Land).		
LAB/BRANCH Biometry Branch		
SECTION		
INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Md. 20892		
TOTAL MAN-YEARS .1	PROFESSIONAL: .1	OTHER .0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The objective is to obtain more accurate information relating to the <u>very low birth weight (VLBW) infant, &lt;1500 grams, for calendar year 1987 than is now available from the United States vital records.</u> This objective will be accomplished by the following: (1) to design and administer a <u>mail questionnaire to mothers of VLBW infants, mothers of all fetal deaths, and a sample of mothers of LBW infants (1,500-2,499 grams) and normal birth weight infants (&gt;2,500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods;</u> (2) to design and conduct <u>telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on mail questionnaires;</u> (3) to develop and conduct procedures for ascertaining from hospital and physician records unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and (4) to prepare and deliver an edited data tape to NICHD. In addition, mortality will be ascertained throughout the first year of life for this birth cohort. This information will help to answer the question: Has there been a reduction in neonatal mortality at the expense of an increase in post-neonatal mortality for these infants?           </p>		

## EPIDEMIOLOGY BRANCH

- |                 |  |
|-----------------|--|
| Z01 HD 00318-06 | A Prospective Study of the Frequency and Duration of Infant Feeding Practices<br>G. G. Rhoads                  |
| Z01 HD 00323-06 | District of Columbia Perinatal Study<br>H. W. Berendes   |
| Z01 HD 00325-05 | Neural Tube Defects and Folate<br>J. L. Mills  |
| Z01 HD 00329-04 | Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.<br>H. W. Berendes                      |
| Z01 HD 00331-03 | Diabetes in Early Pregnancy Project (DIEP)<br>J. L. Mills  |
| Z01 HD 00332-03 | The Risk of Adverse Pregnancy Outcome Following Cervicitis During Pregnancy<br>G. G. Rhoads                    |
| Z01 HD 00333-03 | Congenital Anomalies and In Vitro Fertilization (IVF)<br>J. L. Mills   |
| Z01 HD 00334-04 | Low Birth Weight Across Generations<br>M. A. Klebanoff   |
| Z01 HD 00337-03 | Vomiting during Pregnancy<br>M. A. Klebanoff   |
| Z01 HD 00338-03 | Childhood Nutritional Experience and Subsequent Reproductive Performance<br>M. A. Klebanoff                    |
| Z01 HD 00340-03 | Ethnic Differences in Birth Weight and Length of Gestation<br>P. H. Shiono                                     |
| Z01 HD 00341-03 | Cesarean Childbirth Rates in the U.S.<br>P. H. Shiono  |
| Z01 HD 00342-03 | Dietary Intake of Pregnant Women<br>N. Kurinij   |
| Z01 HD 00343-02 | The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins<br>H. W. Berendes |
| Z01 HD 00344-03 | Long Term Effects of Infant Formulas Deficient in Chloride<br>H. W. Berendes                                   |





- Z01 HD 00345-02 Dietary Supplement and Food Intake in Women of Child-bearing Age  
N. Kurinij
- Z01 HD 00346-02 Time Trends in the Incidence of Biliary Atresia  
M. A. Klebanoff
- Z01 HD 00348-02 The Use of Oral and Other Contraceptive and Congenital Abnormalities  
P. H. Shiono
- Z01 HD 00350-02 A Prospective Study of Congenital Malformations and Maternal Smoking  
P. H. Shiono
- Z01 HD 00351-01 Maternal Recall of Infant Birth Weight  
M. A. Klebanoff
- Z01 HD 00352-01 Studies of Human Immunodeficiency Virus - Related Problems  
G. G. Rhoads
- Z01 HD 00830-05 Child Health Supplement to the 1981 NCHS Health Interview Survey  
M. D. Overpeck
- Z01 HD 00832-03 Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities  
M. D. Overpeck
- Z01 HD 00833-02 Outcomes of Deliveries with IUD Use During Conception  
M. D. Overpeck



## ANNUAL REPORT

October 1, 1985 through September 30, 1986

### Epidemiology Branch EBRP/NICHD

The Epidemiology Branch is involved in major research projects in four areas: 1) low birth weight, 2) teratologic and genetic problems, 3) human immunodeficiency virus infections in mothers and children, and 4) maternal and child health issues in nutrition. In addition the Branch is involved in specific projects on selected other topics in reproductive epidemiology.

#### Low Birth Weight and Perinatal Mortality

The Branch has several projects aimed at exploring the reasons for the differences in low birth weight frequency in different socioeconomic and race groups. Within the District of Columbia field work was completed this year on a case-control study of low birth weight. Cases occurring in six District hospitals accounting for about 85% of such births to District residents were interviewed. Control women with normal birth weight babies were also studied. A variety of demographic, lifestyle and medical factors will be examined to identify particular characteristics in this group of predominantly black women which predispose them to low birth weight.

In another inner city initiative the Branch has collaborated with several private sector organizations in the Better Babies Project, presently a three year effort aimed at reducing the rate of low birth weight infants in a target area in the District of Columbia. Outreach workers will attempt early identification of as many pregnant women as possible in a specific target area of the District and will encourage them to begin prenatal medical care, improve the frequency and total number of their prenatal visits, improve their adherence to health and medical advice and link them with specific interventions designed to reduce prematurity, smoking, and social stress. EBRP is providing recommendations on study design and types of intervention and will be responsible for evaluating the impact of the project. An extensive pilot project has been completed.

Washington, DC and certain other cities have been cited for unusually high rates of infant and perinatal mortality. However, concern with reliability of reporting of early fetal deaths and ambiguous classification between live births and fetal deaths has led to uncertainty in comparing fetal and infant deaths among various jurisdictions. To address this problem Branch staff, working with the Computer Sciences Section and the Biometry Branch, have assembled a data base on perinatal mortality for 59 U.S. cities over the period 1972-1981. These data will allow analysis based on losses after 24 or 28 weeks gestation, which should be more reliable than the usual data on fetal deaths which are based on 20 weeks gestation. By combining late fetal and neonatal deaths it will be possible to produce reasonably comparable perinatal mortality statistics which can be compared among the 59 jurisdictions. Examination of shifts from neonatal



to post-neonatal mortality and correlation with regionalization of care and size of city will be of interest.

The excess of preterm and low birth weight births in lower socioeconomic groups has suggested the possibility that infection might play a causal role. Pathologically defined chorioamnionitis is known to be much more common in preterm than in term births, but the literature relating carriage of particular vaginal or cervical organisms to the onset of labor has been confusing. A major project to examine these issues, funded by CRMC and NIAID, is being largely coordinated by Branch staff. More than 6000 women have been enrolled in five medical centers across the country with eventual enrollment projected to be between 10,000 and 15,000. Vaginal and cervical cultures are being performed on participants during the second trimester of pregnancy. A variety of organisms are being sought. Outcomes are being monitored in terms of subsequent complications of pregnancy, intrapartum events, and perinatal outcome. Women carrying Group B streptococci, Chlamydia trachomatis, and Ureaplasma urealyticum are being invited to participate in a randomized trial of long term erythromycin therapy (1 gram daily) in order to assess its prophylactic effect. To date over 1000 women have agreed to be randomized including 927 with Ureaplasma, 275 with Group B streptococcus and 111 with Chlamydia. (Some women have more than one organism.)

In a related but smaller project the Branch has been investigating the usefulness of cervicitis as a possible marker for preterm birth. Approximately 800 women attending the Johns Hopkins University prenatal clinic have been enrolled in a study including careful observation and photographs of the cervix in the second trimester of pregnancy. Cultures for multiple organisms were also taken. Follow-up of the women has been completed and the data analysis has begun. Results so far suggest that cervical inflammation is difficult to define in a reproducible way, which is likely to make it difficult to use the concept clinically. Within this inner city population Chlamydia colonization was more common in Black (15.4%) than in other (6.9%) women. The association of various aspects of the cervical appearance with organism prevalence is being investigated. Chlamydia was recovered twice as often from women with ectopic change estimated to involve more than 25% of the cervix as in those with less ectopy.

A striking feature of low birth weight is its tendency to recur across generations. For instance, a recent analysis of Perinatal Collaborative Project data showed that women weighing 4-5.9 pounds at birth were 3.5 times more likely to have a low birth weight infant themselves than were women weighing 8 pounds or more at birth. Conversely the women weighing 4-5.9 pounds were only 14% as likely to have a macrosomic infant (greater than 4000 grams). It appears, therefore, that the effect of maternal birth weight on infant's birth weight operates through a shift of the entire distribution rather than specifically on low birth weight. What is not clear from these studies is the extent to which this cross-generational similarity in birth weight is mediated by the rate of intrauterine growth as opposed to the length of gestation. Preliminary evidence from the linked birth certificates of mothers and children born in Tennessee indicates that the rate of intrauterine growth mediates this effect more strongly than does length of gestation. For example, mothers who weighed 2000-2499 grams at birth are nearly 4 times as likely to have a small for gestational age infant compared to mothers who weighed 4000-4499 grams, but only 1.6 times as likely to give birth to a preterm infant. It was not possible to

evaluate the effect of the mother's own gestational age at birth. In order to further determine which of these mechanisms is operating, it will be necessary to acquire data sources from the early 1960's in which both length of gestation and birth weight (and perhaps other confounding factors) were recorded for subjects who can be traced and whose own reproductive performance can be assessed at the present time. Data of this type are being assembled from a health district in Sweden which maintained a low birth weight registry in the 1950's. A request for proposals for a contract to follow-up such a cohort has been issued, and technical review of proposals occurred on June 4-5, 1986. Several investigators, proposing to locate a wide variety of European and American populations, were deemed potentially acceptable.

An analysis of the long term health problems of low birth weight children has been conducted using data from the Child Health Supplement to the 1981 Health Interview Survey. Detailed review of the health status of low birth weight children through 17 years of age showed a consistent pattern of poorer health compared to those born with normal weights. These findings were consistent for such health indicators as limitations of activity, bed days, hospitalizations, number of chronic conditions and parental perception of health status even after controlling for differences in age, race, sex, and maternal education.

Analysis of the Child Health Supplement data also suggests that maternal smoking, which is known to have deleterious effects on intrauterine growth, is additionally associated with compromises in health status of young children. Study of five health status measures for children under 6 years of age indicated a relationship between a mother's smoking habits during pregnancy and the child's subsequent health after birth. The effects seemed to diminish with age for both 'light' and 'heavy' smokers. Younger children (under 3 years) whose mothers were heavy smokers were more likely to be hospitalized or have one or more chronic respiratory conditions than were younger children of lighter smokers or older children at either smoking level.

The Epidemiology Branch is participating in the NICHD Cooperative Maternal Fetal Medicine Unit and Neonatal Intensive Care Unit Networks which have been created to evaluate therapeutic modalities in the perinatal period, especially those relating to low birth weight. The Branch was responsible for establishing the specifications for the Data Center, as well as determination of the format for data entry. Both networks will employ a distributed data entry format. Information will be entered directly on a micro-computer at the study sites eliminating the need for the mail exchange of forms. In addition, the computer will directly aid the collaborating centers in determining eligibility and monitoring protocol compliance. The Epidemiology Branch will provide advice to the data center and the Steering Committee of these two networks on epidemiologic and clinical trials issues. A trial of prophylactic vitamin E is being considered by the Neonatal Network while the Maternal Fetal Network may investigate the advantages of active vs expectant management of post-dates pregnancy.

#### Teratologic and Genetic Problems

The Branch has continued its work on a number of projects relating to the etiology and prevention of congenital malformations. A variety of malformations are more common in births to diabetic women and it is clear that these are



determined (based on their embryology) in the first 6 weeks after conception. The Diabetes in Early Pregnancy Project (DIEP) has recruited women before or within 21 days of conception to identify early pregnancy in 422 diabetic pregnancies and 494 control women. Upon confirmation of pregnancy, the status of the diabetic women was assessed and they were taught to monitor their blood glucose levels at home on a daily basis. Blood was collected on a weekly basis through the first 12 weeks of pregnancy so that metabolic control was closely monitored. Analysis of the relationship between maternal metabolic control during organogenesis and congenital malformations has now begun. The study group has been able to take advantage of the extremely detailed information available on glucose control during the first trimester of pregnancy. Currently seven diabetologists are examining the actual glucose diary data of women producing malformed infants and an equal number of women producing healthy infants. These investigators are not aware of the status of the infants and are attempting to determine whether patterns in the diary data are predictive of malformations. Despite the decreased availability of computer science resources this analysis is proceeding well.

It has long been known that the incidence of neural tube defects (NTD) is subject to some environmental influence which must account for the variation in frequency of this malformation over time and between populations. Recent reports from Great Britain have suggested that periconceptional vitamin supplementation may prevent NTD and have implicated folate more specifically as the active ingredient. The Branch is conducting a case-control study in Illinois and California in cooperation with Northwestern University and the California State Department of Health. We are recruiting neural tube defect cases as well as two groups of control women: those having an ostensibly normal pregnancy as well as a group having a fetus or child with a major medical problem. Cases and controls are being interviewed (by telephone) about three months after birth (or prenatal diagnosis) with special reference to their use of supplementary vitamins around the time of conception. To date we have interviewed 57 neural tube defect cases in Illinois and 145 in California. A total of 92 control subjects have been interviewed in Illinois and 127 in California. Thus far, our ability to identify and locate women whose conceptus has a neural tube defect has been good. Obtaining appropriately matched control subjects has proved somewhat more difficult but is progressing satisfactorily.

A study of congenital malformations and in vitro fertilization is now in the field. Participation rates in both the in vitro fertilization group and control group have been very high. To date approximately 70 children have undergone a detailed malformations examination. Roughly half of these subjects are in vitro fertilization children and half are control subjects. Because the number of subjects available for study is small, an intensive examination is used to identify all malformations. This includes ultrasonography and echocardiography.

The Branch is assisting the CRMC in coordinating a large study to compare the new method of prenatal diagnosis, chorionic villus sampling (CVS), with amniocentesis. CVS is done between 8 and 12 weeks after the last menstrual period and provides results between one and two months earlier than does amniocentesis. The NICHD study involves seven medical centers and will attempt to recruit several thousand women seeking prenatal diagnosis. All patients having CVS at the participating centers will contribute data to assess the accuracy of the procedure, which will be compared to published data in the literature as well as



to the accuracy experience with amniocentesis at the centers. Patients at average obstetric risk who live within one to two hours driving distance of the centers and who have a baseline ultrasound showing a viable pregnancy of 49-90 days gestational age will be asked to participate in the safety study. Many of these women will choose CVS, but a considerable effort is being expended at each center to recruit such women who seek amniocentesis early enough to participate as controls. As of March 15, 1986, 1598 women had been recruited into the safety study (1268 CVS, 330 amniocentesis) and an additional 737 women had been entered in the CVS accuracy study.

Life table analysis will permit examination of loss rates in early pregnancy following CVS and in women (up to 16 weeks) who have had no diagnostic procedure. Following 16 weeks, the comparison would be between CVS and amniocentesis. Follow-up of the course of pregnancy and examination of the newborn infants is also planned. Spontaneous abortion rates in the DIEP participants who had an ultrasound showing a normal, viable pregnancy at about 8 weeks was only about 3%. Since half of these losses could be expected to be aneuploid, a background rate of euploid losses in such women may be as low as 1.5%, explaining the highly favorable CVS statistics which have been reported in the literature to date.

#### Human Immunodeficiency Virus in Mothers and Children

This year the Branch has initiated several activities in the area of human immunodeficiency virus (HIV, HTLV-III) research. The Branch is collaborating with the National Cancer Institute on a study of the effect of HIV infection on pregnant women who belong to high risk groups. Most of these women attend methadone clinics in New York City although an attempt is being made to study women of Haitian background as well. Pregnancy outcome will be compared between women with and without serologic evidence of HIV infection controlling for other risk factors. Some cases of frank AIDS are expected. Vertical transmission of HIV to children is a rapidly growing problem in the United States, but the frequency and implications of such infection are not yet fully known. Clearly, however, a substantial fraction of infected children will develop AIDS.

In addition to these studies of high risk women, the Branch has carried out a sero-epidemiologic survey of HIV positivity in 566 pregnant women attending a regular prenatal clinic in New York City. Several of these women tested positive with the usual ELISA assay and and western blots are now pending. The possibility that false positive rates might be correlated with certain aspects of the obstetric history is being investigated.

There is much public misapprehension about the danger of transmission of AIDS by other than sexual contact or parenteral contamination of needles, etc., and the Branch has collaborated in a study to see to what extent this extends to health care workers. About 1200 employees of a large urban hospital responded to a survey of knowledge, attitudes and beliefs regarding AIDS. Half of the workers believed that AIDS could be spread through ordinary non-sexual contact while 20% said it could be spread by a cough or sneeze. One out of six workers believed that AIDS was more common in hospital workers than in others. 50% said they would wear a gown or mask in the room of an AIDS patient while 25% reported that they avoided public places like swimming pools because of fear of AIDS transmission. Accurate knowledge about AIDS and its transmission was highly

significantly correlated with low anxiety and a willingness to work with AIDS patients.

### Nutrition

The Branch has continued to be involved in several projects relating to nutrition during pregnancy and childhood. Because it is well known that relatively few American women breast feed their infants for the recommended four to five months, a study of black and white women has been carried out to investigate the underlying reasons for this. Approximately 1200 primiparae were interviewed during the first few days postpartum to ascertain their infant-feeding plans and the factors which led them to choose breast feeding or bottle feeding. These women were followed through the first year with a series of interviews to ascertain when they actually stop breast feeding and their reasons for stopping. Data from the hospital interviews and from interviews conducted at one, three, seven, and twelve months post-partum have currently been edited and data analysis will begin this year. Ethnic differences in the rate of breast feeding are evident with 85% of white women breast feeding at birth compared to only 49% of black women giving birth in the three hospitals selected for the study. Besides race, low breast feeding rates were also associated with having less than a high school education, living with grandparents, being unmarried, and not attending childbirth classes. Maternal education, however, was substantially the most important of 16 predictors of infant feeding practice. In the full analysis of the present data, factors affecting duration of breast feeding will be examined. Approximately 28% of women stop breast feeding by one month, 52% by three months and 80% by seven months.

EBRP has continued to be involved in the analysis of the Study of Infant Feeding among Bedouin Women in the Negev Desert of Israel. Initial analysis of these data has revealed a marked seasonal variation in births in this population with the highest rates of birth occurring in the winter months and lowest rates in the summer. Variation is as much as 30% between summer and winter. Since this population does not practice contraception the reason for this marked seasonal variation is not clear and is the subject of further careful data analysis. A strong relationship has emerged between health complications at the time of birth and subsequent infant feeding choice. Women who experience serious complications during pregnancy or are delivered by Cesarean section resort primarily to bottle feeding. Also children who are low birth weight or who have severe malformations or major medical problems tend to be bottle fed. Bedouin women traditionally practice a 40 day rest period after the birth of their children, during which help is provided by relatives for the domestic chores. Analysis of infant feeding practices at two months clearly demonstrates a strong correlation between duration of help and exclusive breast feeding. Other analyses are in progress to more fully document the relationship between changes in infant feeding practices, westernization, and health of the infants.

In an analysis of data from the National Health and Nutrition Examination Survey the relationship between dietary adequacy and food supplement use has been explored. Among 3,227 non-pregnant women aged 15-41, 25% used dietary supplements regularly and 67% of these consumed some form of multivitamin. Women using food supplements consumed significantly more dietary protein, phosphorus, iron, potassium, thiamin, and niacin than did non-users of supplements. Dietary differences remained after adjusting for socioeconomic, geographic, and



anthropometric differences between the supplement usage groups. It appeared that supplement users were the individuals who least needed such supplements.

In collaboration with the University of Maryland a study was conducted among 51 college students who were asked to rate 23 foods representative of the four food groups. A multidimensional scaling analysis was used, and it was found that respondents grouped foods by criteria other than nutrient composition. Results indicate that consumers' criteria for grouping foods should be considered when developing food guides for nutrition education.

Childhood nutrition is generally recognized to have some influence on the onset of puberty. It is well known that large and especially obese children tend to go into puberty early while children who are sick or malnourished tend to have later onset of puberty. There is some evidence that the average age at menarche has decreased since the 17th and 18th centuries in western countries, a change which is widely attributed to improved health and nutrition. As reported last year an exploratory study has been carried out on a cohort of 80 boys in Berkeley, California, whose nutritional intake and anthropometric measurements have been carefully documented since infancy. These boys were assessed for pubertal status at age 14 and the findings were related to their early nutritional measures. No differences in nutrient intake could be documented between the boys who were at an advanced stage of puberty as compared to those who were just starting. On the other hand the more advanced boys were found to have been heavier as infants and throughout childhood. Initially, the extra weight appeared to be attributable to an increase in length and lean body mass as well as to a slight increase in skinfold thickness. Further analysis this year revealed that lean body mass was most highly correlated with the timing of puberty. These data suggest that in boys growth in early life and particularly muscle mass are related to the onset of puberty. It is not yet clear if these are genetically mediated and if so whether endocrine factors play an important role.

The EBRP continues to pursue the Congressionally mandated study of the long-term effects of exposure to chloride-deficient infant formula. This year, the Program initiated three pilot studies relevant to the design of the final study. The first pilot, carried out in Memphis, showed that the test battery as developed by the EBRP is adequate and feasible but that appropriate controls cannot be identified and recruited for study participation from the offices of the pediatrician of the cases. The pilot study begun in Boston continues to examine the relationship between defective formula ingestion and the development of overfocusing syndrome. The pilot done in Sarasota, Florida, was a population based study of exposed vs control children. This study demonstrated a significant ( $p=.05$ , one tailed test) difference between exposed and control children in general cognitive abilities and quantitative abilities and a non-significant but suggestive difference between exposed and unexposed children in perceptual abilities. These results are consistent with the majority of the findings demonstrated in the Clinical Center study done in 1980 and 1982.

### Other Topics

In cooperation with the American College of Obstetricians and Gynecologists (ACOG) the Branch has completed an analysis of a survey of U.S. hospitals conducted by ACOG concerning updated information on Cesarean childbirth.



Questionnaires were mailed to 538 hospitals and 87% responded. In 1979, 2.1% of women with a prior cesarean delivery were given a trial of labor. By 1984, the rate increased four-fold to 8.0%. Over 50% of the trials of labor resulted in a successful vaginal delivery. However, the fraction of hospitals with no trials of labor remains high (54%). The cesarean birth rates increased from 14.1% in 1979 to 19.0% in 1984. Fetal distress accounted for a larger proportion of primary cesareans in 1984 (21%) as compared to 1979 (14%). The observed increase in the rate of trial of labor does not appear to be large enough to stem the rising cesarean delivery rate. The present survey and other national surveys provide no evidence that the cesarean delivery rates are leveling off or decreasing.

In a methodological study data from the 1981 Child Health Supplement, Health Interview Survey were assessed for completeness of reporting of complications of delivery by making comparison to other national data sources. The reasons for Cesarean sections as reported by mothers appeared reliable and can presumably be used in analysis of health outcomes. Compared to hospital discharge reports or a follow-back natality survey, maternal reporting was not complete for other complications of labor and delivery.

A study of nausea and vomiting of pregnancy was carried out based on the Collaborative Perinatal Project. Information on vomiting was recorded at each prenatal visit in that study. As reported last year the frequency of vomiting was common, affecting 37% of women during weeks 1-4, reaching a peak of 44% during weeks 5-8 and descending to 29% by weeks 13-16. Miscarriage and stillbirth was less common among women who vomited (relative risk = .70,  $p=.002$ ) than among those who did not. Women who experienced vomiting carried their pregnancies an average of 1.5 days longer ( $p<.001$ ) and were 17% less likely to deliver before 37 weeks ( $p=.004$ ). Overall, there was evidence that vomiting was a normal occurrence in the first trimester and, if not excessive, compatible with outcomes which were slightly more favorable than among non-vomiters. This analysis was extended this year to examine the incidence of malformations among pregnancies with and without vomiting. No category of malformations was found to be more common among women experiencing vomiting. These findings are of interest in relation to levels of particular hormones which are believed to be responsible for nausea and vomiting of early pregnancy, and in relation to the potential teratogenicity of anti-emetic drugs.

Work on the characteristics of 350 live births occurring to women in the Kaiser-Permanente Birth Defects Study who conceived while an IUD was in place has been done collaboratively with young investigators from the University of North Carolina and the National Center for Health Statistics. Preliminary findings reinforce the expected early gestational loss but show no difference in birth weight or gestational age of the surviving infants. Comparisons by maternal characteristics and for use of other and no contraceptives are underway. The study was performed during the period of the widest use of various types of IUD's. Further analysis of the outcome of their use, particularly for live born children not normally studied, are of interest.

## Presentations:

Shiono, P.H. Congenital malformations and maternal smoking during pregnancy. Presented at the International Conference on Smoking and Reproductive Health, San Francisco, CA, October, 1985.

Mills, J.L. Current concepts in detection and prevention of congenital malformations and developmental abnormalities. Presented at Epidemiologic Perspectives on Women's Health, University of Pittsburgh, PA, October, 1985.

Mills, J.L. Alcohol effects on Pregnancy. Presented at the Second International Medical Conference on Maternal and Fetal Health in Pregnancy, University of Rome, La Sapienza, Fiuggi, Italy, October, 1985.

Shiono, P.H. Ethnic differences in preterm delivery. Presented at the annual meeting of the American Public Health Association, Washington, DC, November, 1985.

Mills, J.L. Pregnancy and fetal malformation in diabetes. Presented at the Juvenile Diabetes Foundation International - World Conference on Diabetes Research, Monte Carlo, Monaco, November, 1985.

Overpeck, M.D. Low birth weight and child health status, 1981. Presented at the annual meeting of the American Public Health Association, November, 1985.

Rhoads, G.G. Nutrient intake and chronic disease: Some methodological issues. Presented to the Nutrition Coordinating Committee, National Institutes of Health, December 5, 1985.

Mills, J.L. Diabetes mellitus in pregnancy. Presented at the Walter Reed Army Medical Center, Washington, DC, January, 1986.

Klebanoff, M.A. The epidemiology of low birth weight. Presented at the Johns Hopkins University School of Public Health, Baltimore, MD, February, 1986.

Rhoads, G.G. Lp(a) lipoprotein as a risk factor for myocardial infarction. Presented to the 26th Annual Conference on Cardiovascular Epidemiology, San Francisco, CA, March 3-5, 1986.

Klebanoff, M.A. Studies on low birth weight. Presented at the University of Maryland, Baltimore, MD, April, 1986.

Klebanoff, M.A. Mother's birth weight and disproportionate fetal growth. Presented at the annual meeting of the Society for Pediatric Research, Washington, DC, May, 1986.

Mills, J.L. Does moderate alcohol consumption during pregnancy cause malformations? Presented at the annual meeting of the Society for Pediatric Research, Washington, DC, May, 1986.

Mills, J.L. Congenital malformations in diabetic pregnancy: An overview. Presented at the Council on Pregnancy and Council on Youth, American Diabetes Association, Anaheim, CA, June, 1986.

Rhoads, G.G. Risk factors for Chlamydia in a pregnant population. Presented at the annual meeting of the Society for Epidemiologic Research, Pittsburgh, PA, June, 1986.

Klebanoff, M.A. How well do mothers recall the birth weight of their children? Presented at the annual meeting of the Society for Epidemiologic Research, Pittsburgh, PA, June, 1986.

Nugent, R.P. Risk factors for cervicitis and Chlamydia trachomatis in an inner city population. Presented at the Sixth International Symposium on Human Chlamydial Infections, Sanderstead, Surrey, England, June, 1986.

Kurini, N. Analysis of the four food groups using multidimensional scaling. Presented at the annual meeting of the Society for Nutrition Education, Washington, DC, July, 1986.

Rhoads, G.G. Risk factors for cervicitis in a pregnant population. Presented at the annual meeting of the American Public Health Association, Las Vegas, NV, September, 1986.

Kurini, N. Breast feeding in a biracial population. Presented at the annual meeting of the American Public Health Association, Las Vegas, NV, September, 1986.



NICHD ANNUAL REPORT

October 1, 1985 through September 30, 1986

Epidemiology Branch

Publications

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## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00318-06 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Prospective Study of the Frequency and Duration of Infant Feeding Practices

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Natalie Kurinij Nutritionist EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD

## COOPERATING UNITS (if any)

Computer Sciences Section, EBRP, NICHD (E.E.Harley); Biometry Branch, EBRP, NICHD (D.W.Derman); Johns Hopkins Univ., Baltimore, MD (M.R.Forman); G.W.Univ. Medical Center, Washington, DC (M.Edwards); Univ. of Md., College Park, MD (M.L.Axelson).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

.45

## PROFESSIONAL:

.05

## OTHER:

.40

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although breastfeeding is generally recognized as the optimal way to feed infants through the first 4-5 months, it is well known that many American women nurse their babies for much more limited periods or not at all. In this prospective study characteristics associated with choice and duration of breast feeding are being investigated. The specific objectives of the study are: (1) to provide detailed information on the change in the infant-feeding pattern over time; (2) to investigate the underlying meaning of the milk insufficiency syndrome; (3) to investigate the relation between maternal employment and choice and duration of breast feeding; (4) to determine the sociocultural differences in infant feeding between two ethnic groups. Approximately 1200 women having their first child in one of three hospitals in the Washington, DC, area were interviewed with respect to factors that may have influenced their plans for infant feeding. Data collection was completed in April, 1986, and data cleaning, editing and file building were completed in August, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00323-06 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

District of Columbia Perinatal Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Heinz W. Berendes Director EBRP/NICHD

COOPERATING UNITS (if any)

Epidemiology Branch, EBRP, NICHD (L.C.Cooper); Biometry Branch, EBRP, NICHD (D.W.Derman).

LAB/BRANCH

Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). Approximately 75% of the data has now been keyed. NICHD has completed edit checks on 60% of the data. Upon completion of the data being keyed and edited, manual editing will begin. It is expected that analysis will begin in the fall, 1986.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 HD 00325-05 EB

PERIOD COVERED  
October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type Do not exceed the space provided.)

The Epidemiology Branch (EBRP) is conducting a case-control study in Illinois and California to determine whether the use periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect in either state will be ascertained through perinatal networks, vital records, and other sources and will be matched to two controls on maternal race and geographic locale. One control will be a mother with a normal pregnancy, and the other the mother of an infant or fetus with a major health problem. Cases and controls will be interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. The study design, personnel hiring, and forms development have now been completed and case identification is now underway. Field work has been contracted to the Department of Health, State of California and to Northwestern University in Illinois. To date approximately 225 cases and 400 control subjects have been identified and interviewed. Ascertainment of cases has been excellent in Illinois where the population is relatively small. Ascertainment has been good in California where, despite the fact that some cases are not identified, the total number of subjects obtained has been highly satisfactory.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00329-04 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Heinz W. Berendes

Director

EBRP/NICHD

## COOPERATING UNITS (if any)

Epidemiology Branch, EBRP, NICHD (M.Overpeck and L.C.Cooper); Greater Washington Research Center, Washington, DC (J.Maxwell); Better Babies Project, Washington, DC (D.Coates).

## LAB/BRANCH

Office of the Director, EBRP

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.4

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Better Babies Project (BBP) is a three-year research and demonstration effort to reduce the rate of low birth weight and associated infant mortality and illness in a specific high risk area of the District of Columbia. The Project will attempt to identify all pregnant women in a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findings a number of revisions have been made in the forms and interventions. These revised forms and interventions are presently being developed and piloted. It is expected that a four year trial of the project will begin September, 1986.

Evaluation of the project will be provided by the National Institute of Child Health and Human Development, Epidemiology and Biometry Research Program (EBRP).

NICHD has let out two contracts for the Better Babies Project to assist with the evaluation. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, will be providing us information on all pregnant women delivering in the District of Columbia during the period of the project. Levin and Associates in Rockville, Maryland, under contract, is providing data support in the evaluation of the impact of the BBP.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00331-03 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diabetes In Early Pregnancy Project (DIEP)

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

## COOPERATING UNITS (if any)

Cornell Univ.Med.Center, NY, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ. of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ.of Washington, Seattle,WA (R.Knopp).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

0.5

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The Diabetes in Early Pregnancy Project has the following objectives: 1) To examine the relationship between maternal diabetic control during organo-genesis and malformations in the offspring. To identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) To compare early fetal loss rates in women with diabetes and control subjects. All of the forms in the DIEP have now been accounted for. All forms and all diary data in the first trimester have now been computerized. Most edits have been run on most forms. As noted in the previous report, the change from in-house to contract computer sciences services has delayed examination of the DIEP data. An outside support service contract for computer is not yet in place. However, some analysis has been begun through a professional services contract. Because of the limited personnel time available, only the malformations question can be addressed at this time. Currently, metabolic data including extensive (181,000 glucose values) diary data are being examined to determine the relationship between metabolic control and malformations. Because of the delay in obtaining contract computer services it is difficult to estimate when analyses can be completed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00332-03 EB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Risk of Adverse Pregnancy Outcome Following Cervicitis during Pregnancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert P. Nugent Epidemiologist EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

Johns Hopkins University, Baltimore, MD (B.F.Polk, L.Berlin).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All eligible women (age 18 and older) seen in the obstetric clinic at Johns Hopkins University between November 1983 and January 1985 who agreed to participate had their cervix evaluated for signs of inflammation. In addition cultures were taken for a number of aerobic and anaerobic organisms and a sample of cervical mucus was evaluated for the presence of inflammatory cells. The women were interviewed to obtain information on a number of risk factors related to preterm and low birth weight delivery. The women were then followed to delivery to evaluate the effect of cervicitis on preterm or low birth weight delivery. Approximately 800 women participated in this study.

Data tapes have been provided by Johns Hopkins and data editing programs have been developed. Analysis began in August 1985 with articles to be submitted for publication as soon as possible. Preliminary findings were presented at the Sixth International Symposium on Human Chlamydial Infections and the Society for Epidemiologic Research in June 1986.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00333-03 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Congenital Anomalies and In Vitro Fertilization (IVF)

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

## COOPERATING UNITS (if any)

Director, EBRP (H.W.Berendes); Computer Sciences Section, EBRP (E.E.Harley).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

.1

## PROFESSIONAL:

.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro fertilization has become an increasingly popular method of conception over the past few years. To date no formal study of infants conceived in vitro has been conducted to determine if they are at increased risk for congenital malformations. Dr. Mills and the Epidemiology Branch are conducting a historical prospective study of infants which have been conceived in vitro and matched controls to determine whether in vitro fertilization carries an increased risk for congenital malformations. The Eastern Virginia Medical School, Norfolk, VA, is serving as study and data center for this project (Dr. Fred Wirth, Principal Investigator). Extensive investigations are performed on each in vitro fertilization subject and control subject. These include physical examination, intracranial ultrasound, echocardiography, electrocardiography, and abdominal ultrasound. This contract is progressing very well and to date 62 subjects have been examined out of a total of 160 potential participants. Well over 90% of potential participants in both the IVF and control groups have agreed to participate. We anticipate that this contract will be completed on schedule or perhaps even before.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00334-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

Office of the Director, EBRP, NICHD (H.W.Berendes); University Hospital, Uppsala, Sweden (O.Meirik); Centers for Disease Control, Atlanta (R. Yip).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.55

PROFESSIONAL:

.55

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The original description of the association of maternal and infant birth weights was followed by the description of the association between increased maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Compared to mothers who weighed 8 pounds or more at birth, mothers who weighed 6 to 7.9 pounds were only half as likely, and mothers who weighed 4-5.9 pounds were 15 percent as likely to give birth to a macrosomic infant. In order to further investigate the specific effect of maternal birth weight on disproportionate fetal growth, other fetal growth parameters were studied, using data from the Collaborative Perinatal Project. Infants of low birth weight mothers were shorter than infants of larger mothers, but were normally proportioned. Results of these investigations were presented at the 1986 meetings of the Society for Pediatric Research.

In a related study, birth certificates of infants born in Tennessee between 1979 and 1984 were matched with those of their mothers, who were born in Tennessee between 1959 and 1966. The association between maternal and infant birth weight was confirmed. In addition, it was shown that low maternal birth weight carried a greater risk for the delivery of a small for gestational age infant than for a preterm one. Results of this investigation will be presented at the 1986 meetings of the American Public Health Association.

An ongoing study involves abstraction of the birth records of a sample of women born in a region of Sweden during the 1950's. The reproductive histories of these women will be traced through the birth registry. Finally, review of proposals occurred in June 4-5, 1986 for a contract involving the establishment and follow-up of a cohort of women whose own intrauterine and perinatal experience has been previously documented. Reproductive outcome of these women will then be determined.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00337-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vomiting During Pregnancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD  
James L. Mills Research Medical Officer EB/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

.2

PROFESSIONAL:

.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Vomiting during pregnancy has been described since 2,000 B.C., but few studies have attempted to describe its epidemiology. First trimester registrants in the Collaborative Perinatal Project were screened for the presence of vomiting. Vomiting was more common in primigravidas, young women, heavy women, non-smokers and women with less education. The absence of vomiting placed a woman at increased risk of fetal loss. There was a modest protective effect on preterm delivery, and no effect on the incidence of low birth weight. Adjustment for confounders by multiple logistic regression confirmed these associations.

The effect of vomiting in the absence of use of antiemetic drugs on the incidence of congenital malformations was also examined. This analysis used women who registered during the first 20 weeks of pregnancy. Classification of malformations began with the data of Myrianthopoulos and Chung, but was modified to reflect current concepts of the pathogenesis of malformations. Antiemetic drugs were defined as those classified by Heinonen, Sloane and Shapiro as "antinausants, antihistamines and phenothiazines" and were also listed in the 1965 Physicians Desk Reference as being indicated for nausea, motion sickness, hyperemesis or vomiting. It was found that vomiting was unassociated with either major malformations, deformations, hernias or minor malformations. Adjustment for race, maternal age, gravidity, infant sex, study center and antiemetic use did not substantially alter the odds ratios.

This project was terminated during FY'86.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00338-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Childhood Nutritional Experience and Subsequent Reproductive Performance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

Columbia University, New York, NY (Z.A.Stein, L.H.Lumey).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.05

PROFESSIONAL:

0.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Girls born during the Dutch famine of 1944-45 are known to have been growth retarded as a direct result of maternal starvation, although final adult height was not reduced. Girls age 12-14 during the famine were permanently stunted. The subsequent reproductive experience of several cohorts of women who were of different ages during the famine was determined. These cohorts include women who were conceived and born before the famine, conceived before and born during the famine (subdivided into exposed third trimester only and exposed second and third trimester), conceived before and born after, conceived during and born after, and conceived and born after the famine.

Of all singleton deliveries in 1960-1983 at the Wilhelmina Gasthuis in Amsterdam, there were 1808 to primiparous women born between January 1, 1944, and June 30, 1946. Adult height and prepregnant weight did not differ by birth cohort. In spite of this, the infant birth weight varied by birth cohort of the mother. Mean infant birth weight was highest among mothers conceived and born before the famine. It progressively decreased to over 180 grams less among women exposed to famine during their own first and second trimesters. Women conceived and born after the famine had infants whose birth weights were not significantly different from those conceived and born before the famine.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00340-03 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ethnic Differences in Birth Weight and Length of Gestation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

Others: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD  
George G. Rhoads Branch Chief EB/EBRP/NICHD  
Natalie Kurinij Nutritionist EB/EBRP/NICHD  
Anne Willoughby Epidemiology Staff Fellow EB/EBRP/NICHD

## COOPERATING UNITS (if any)

Office of the Director, EBRP, NICHD (H.W.Berendes).

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.45

## PROFESSIONAL:

0.45

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

## A. Kaiser Study

Data from the Kaiser-Permanente Birth Defects Study are being used to evaluate differences in birth weight and gestational age among four different ethnic groups. The ethnic groups included in the Kaiser study are Whites, Hispanics, Blacks and Asians. In addition to studies of ethnic differences, we have evaluated the effects of smoking and drinking during pregnancy on preterm births.

## B. Ethnic Differences in Lifestyle, Psychosocial Factors and Medical Care During Pregnancy.

A Contract to obtain a quantifiable description of behavior and lifestyle differences among pregnant women of different ethnic groups which are known to differ in their rates of low birth weight is being developed. The overall goal of this project is to define previously undescribed risk factors affecting birth outcome from pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, and Whites. The work scope of the contract includes development of an extensive questionnaire by a multidisciplinary team of experts, pretesting of the interview instruments, interviewing pregnant women from the five groups noted above, and preparing an edited data tape of all responses. It is anticipated that the contract will be awarded during the summer of 1986.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00341-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cesarean Childbirth Rates in the U.S.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

American College of Obstetricians and Gynecologists (ACOG), Washington, DC  
(W.H.Pearse).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.15

PROFESSIONAL:

0.15

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A nationally representative survey has been completed by ACOG to determine the cesarean childbirth rates in the U.S. and current hospital policies regarding cesarean childbirth. Members of the EB staff acted as consultants to ACOG to assist in the design of the survey, sampling methodology and analysis of the results. The purpose of the survey was to evaluate changes since 1979 in rates of cesarean delivery and trial of labor after a previous cesarean. Questionnaires were mailed to 538 hospitals and 87% responded. In 1979, 2.1% of women with a prior cesarean birth were given a trial of labor. By 1984, the rate increased four-fold to 8.0%. Over 50% of the trials of labor resulted in a successful vaginal delivery. However, the fraction of hospitals with no trials of labor remains high (54%). Cesarean birth rates increased from 14.1% in 1979 to 19.0% in 1984. Fetal distress accounted for a larger proportion of primary cesareans in 1984 (21%) as compared to 1979 (14%). The observed increase in the rate of trial of labor does not appear to be large enough to stem the rising cesarean delivery rate. The present survey and other national surveys provide no evidence that the cesarean delivery rates are leveling off or decreasing.

In addition to this survey, NICHD had obtained information from the Commission on Professional Hospital Activities on national rates of cesarean childbirth that will be comparable to information obtained by the institute in 1980.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00342-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary Intake of Pregnant Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Natalie Kurinij Nutritionist EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

Biometry Branch, EBRP, NICHD (B.I.Graubard).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.05

PROFESSIONAL:

0

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pregnant women are at increased risk of malnutrition due to the increased nutrient demands of pregnancy. Nutrient intake during pregnancy is being assessed using data from the NHANES I survey. The dietary patterns of a national sample of pregnant women is being evaluated to determine differences in nutrient intake and food frequency during each trimester of pregnancy. Nutrient intake during pregnancy is being compared to the nutrient intake of nonpregnant women of childbearing age and to the recommended dietary allowances.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-00343-03 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.W. Berendes Director, EBRP NICHD

## COOPERATING UNITS (if any)

Department of International Health, Johns Hopkins University, Baltimore, Md. (M.R. Forman); Biometry Branch, EBRP, NICHD, (B. Graubard); Computer Sciences Section, EBRP (D. Towne); Ben Gurion Univ. on the Negev, Beer Sheva, Israel (L. Naggan)

## LAB/BRANCH

Office of the Director, EBRP

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

.3

## PROFESSIONAL:

.2

## OTHER:

.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is concerned with infant feeding practices among Bedouin tribes who are residing in the Negev. Objectives include: the evaluation of changes in infant feeding practices during the first year of life on physical growth of children and on gastrointestinal and respiratory diseases especially episodes of sufficient severity to result in hospitalizations during the first year of life.

Data have been obtained on about 5,000 mother-infant pairs. About half of the sample was identified at birth and a subsample followed for a period of from 5-8 months. Another sample of children was identified at 6 months of age and infant feeding histories were obtained retrospectively prior to 6 months with a prospective follow-up of this sample to 18 months of age.

The data collection for this project is complete and the information is computerized. Analysis on choice of infant feeding practices at birth clearly reveal that bottle feeding in this population is highly related to pregnancy complications and to the condition of the child at birth including low birthweight. Mothers with various complications, those undergoing Ceasarean Section and those having low birth weight children or children with congenital malformations and/or serious problems in the newborn nursery tend to bottle feed their children. Continuation of breast feeding to age 2 months is markedly increased if women obtained substantial help in handling daily chores in and outside the house.

Three papers from the study were presented at a national meeting and one at an international maating during the past year.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00344-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Long Term Health Effects of Infant Formulas Deficient in Chloride

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Heinz W. Berendes

Director

EBRP/NICHD

COOPERATING UNITS (if any)

Epidemiology Branch, EBRP, NICHD (A.Willoughby, G.Rhoads); Biometry Branch, EBRP, NICHD, (B.I.Graubard).

LAB/BRANCH

Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS

1.5

PROFESSIONAL:

1.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a Congressionally mandated study to determine whether the children exposed to chloride deficient formula in 1978 and 1979 may have suffered some long-term effects which may be expressed in delayed motor and mental development or decreased school performance.

This project is currently receiving support services from SAIC, Inc. (formerly JRB Associates). The activity of the past year has centered on 1) identifying subjects eligible for study as cases, 2) investigating the potential problems exposed children may have, 3) defining the test battery that cases and controls will undergo, 4) a pilot test of all study procedures, 5) a pilot test of a complete battery of potentially useful neuropsychological tests, and 6) the completion of a population-based study of children exposed to chloride deficient formula and their appropriate controls. Case finding has consisted of surveys of pediatric nephrologists, pediatricians in several states and a systematic CPHA search. Problem identification has consisted of the careful review of several hundred potential case records, a literature review, discussion with physicians who have treated cases, and a review of problems as described by parents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00345-02 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary Supplement and Food Intake in Women of Childbearing Age

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Natalie Kurinij Nutritionist EB/EBRP/NICHD

Others: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

Biometry Branch, EBRP, NICHD (B.I.Graubard)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

.05

PROFESSIONAL:

.01

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The relationship between dietary adequacy and food supplement use was examined in 3,227 nonpregnant women aged 15 to 41 from the first National Health and Nutritional Examination Survey. Twenty-five percent of women used dietary supplements regularly, and 67 percent of these consumed some form of multi-vitamin. Supplement users were of a higher income and education, were more often white, had a leaner body composition, and were more likely to reside in the Western U.S. as compared to nonusers. Caloric intake between supplement usage groups was similar. However, supplement users consumed significantly more dietary protein, phosphorus, iron, potassium, thiamin, and niacin than did nonusers. A considerable portion of both usage groups had intakes below 50 percent of the Recommended Dietary Allowances for calcium, iron, vitamin A, and vitamin C; however, a significantly greater proportion of supplement nonusers had low intakes of iron and vitamin C. Food supplement users were found to consume a more nutrient dense diet and may be the individuals who least need supplements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 HD 00346-02 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Time Trends in the Incidence of Biliary Atresia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

Others: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

Case Western Reserve University (B.Chatterjee)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.05

PROFESSIONAL:

0.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project will gather information on all cases in a well defined geopolitical area for approximately 10 years, and birth certificates will be obtained. Cases will be compared to the other births in the area for evidence of changes in incidence and clustering.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00348-02 EB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Use of Oral and Other Contraceptives and Congenital Abnormalities

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

## COOPERATING UNITS (if any)

Hebrew University of Jerusalem, Jerusalem, Israel (S.Harlap)

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

.10

## PROFESSIONAL:

.10

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Congenital malformations were observed in 33,545 newborns whose mothers had been questioned during pregnancy about contraceptives used around the time of conception. There were 597 babies (17.8/1,000) with major malformations and 4,046 (120.6/1,000) with minor ones. The 8,522 offspring of mothers who had used oral contraceptives (OC) prior to conception showed 17.2/1,000 major malformations compared with rates of 15.0 and 20.1/1,000 in the groups who had used other methods or no birth control prior to conception. There was no evidence for an increased risk of malformations in women conceiving within one month of stopping OC. There were 850 babies exposed to OC in utero and the ratio of observed to expected cases of major malformations was 1.24 (n.s.) if the mother was a nonsmoker and 2.98 ( $p = .028$ ) if the mother smoked one pack or more of cigarettes daily. There were no significant changes in malformation rates following failures of intrauterine devices, spermicides or rhythm contraception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00350-02 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Prospective Study of Congenital Malformations and Maternal Smoking

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

Others: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.1

PROFESSIONAL:

.1

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The relationship between smoking during pregnancy and congenital malformations was studied in prospective studies of 33,434 births in the Northern California Kaiser-Permanente Birth Defects Study and 55,933 births in the Collaborative Perinatal Project (CPP). 28.4% of women were smokers in the Kaiser population and 47% smoked in the CPP population. The odds ratio for smoking during pregnancy and major malformations in Kaiser was 1.00 (95% C.I. 0.8-1.2) and the odds ratio for minor malformations was 0.9 (0.8-0.9) (p .001). The relationship between smoking and 54 individual malformations was evaluated in the Kaiser population. Statistically significant positive associations were observed for ventral hernias (10.1 (1.1-91)) and 'other major gut abnormalities' (12.6 (1.5-108)). However, for each malformation the estimates were based on only one unexposed case. Significant negative associations were found for ventricular septal defects (0.5 (0.2-1.0)), hydroceles (0.7 (0.6-0.9)), clubfoot (0.7 (0.6-0.9)), pigmented nevi (0.7 (0.6-0.9)), hemangiomas (0.8 (0.7-0.9)), and Down syndrome (0.2 (0.1-0.9)). To determine if the findings noted above were an artifact of multiple comparisons, 7 of these 8 malformations were tabulated by smoking status for women in the CPP. All but one of the associations were not confirmed in the CPP. The association between smoking and hemangiomas in the CPP was 0.8 (0.6-1.0) (p=0.03). The prevalence of hemangioma at birth was 3.9% in the Kaiser population and 0.5% in the CPP. Adjustment for ethnicity strengthened this association in both data sets. We conclude that smoking is unlikely to be responsible for an increase in malformations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00351-01 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Maternal Recall of Infant Birth Weight

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark A. Klebanoff Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Low birth weight is a major public health problem and birth weight has been the subject of numerous epidemiologic studies. Infant birth weight has almost exclusively been abstracted from medical records or birth certificates, and there has been little attempt to assess the accuracy of maternal recall of birth weight. In this project, the birth weight of a mother's first child, as stated by the mother at registration for prenatal care during her next pregnancy, was compared to the birth weight as given in that child's medical records. There were 7521 pregnancies studied.

Overall, only 4.2% of women were unable to state a birth weight for their most recently born child. Blacks, women of higher parity, younger women, and women with less education were less likely to state a birth weight for their most recently born child. Mothers were less likely to state a birth weight for infants less than 1000 grams and infants who did not survive the first 48 hours.

Among those who did state a birth weight (n=5582) the errors in birth weight was unbiased with respect to most factors, but there was a slight tendency to overstate the birth weight of small infants and understate large ones. Low birth weight was identified quite accurately. The sensitivity of reported low birth weight was 96%, and the specificity of reported normal birth weight was 98%. It is concluded that among reproductive age women, recalled birth weight can reasonably be substituted for medically verified birth weight.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00352-01 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders )

Studies of Human Immunodeficiency Virus - Related Problems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George G. Rhoads Branch Chief EB/EBRP/NICHD

Others: Anne Willoughby Senior Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

The EBRP has developed three ongoing initiatives related to HIV and AIDS:

A. An investigation of the knowledge, attitudes and behavior of health care workers toward AIDS and AIDS patients.

B. A seroepidemiologic study of pregnant women in New York City looking for evidence of HIV infection.

C. Collaboration with the National Cancer Institute on a study of the natural history of retrovirus infection in pregnant women and their offspring.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00830-05 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Child Health Supplement to the 1981 NCHS Health Interview Survey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary D. Overpeck Health Statistician EB/EBRP/NICHD

Other: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

Office of the Director, EBRP, NICHD (H.W.Berendes); Biometry Branch, EBRP, NICHD (H.J.Hoffman); Division of Health Interview Survey, National Center for Health Statistics (G.Hendershot and A. Moss)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.3

PROFESSIONAL

.2

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )

This project provides data on a nationwide sample of 17,000 children of indices of child development, childhood morbidity, school performance and behavior. It establishes normative ranges for the U.S. as well as demonstrating the long-term consequences of perinatal and early childhood risks. The survey was conducted by the National Center for Health Statistics in collaboration with NICHD and others in 1981 as part of the continuous National Health Interview Survey. Final public use tapes were released by NCHS in late 1984. Collaborative NICHD/NCHS publications have been prepared on the current health status of low birth weight children and the health outcome of children whose mothers smoked during pregnancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00832-03 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PIs:	Leslie C. Cooper	Nurse Epidemiologist	EB/EBRP/NICHD
	Mary D. Overpeck	Health Statistician	EB/EBRP/NICHD
Others:	George G. Rhoads	Chief	EB/EBRP/NICHD

COOPERATING UNITS (if any)

Office of the Director, EBRP, NICHD (H.W.Berendes); Biometry Branch, EBRP, NICHD (H.J.Hoffman); Computer Sciences Section, EBRP, NICHD (E.E.Harley); Mortality Statistics Branch, DVS, NCHS (H.Rosenberg).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.5

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study reviews changes and differences in perinatal mortality for similar populations over a period of rapid change in technology and medical management of high risk pregnancies.

It will explore whether high rates of neonatal mortality in certain cities can be explained by shifts in mortality from the late fetal to the neonatal period and will compare differences in perinatal experience according to race and city size. The approach will be a secondary analysis of data sets provided by the National Center for Health Statistics based on 100 percent reporting of perinatal deaths. Review of fetal death rates for the periods 20-27 and 28+ gestation and of neonatal deaths for the periods 0-7 and 8-28 days will be used to examine potential reporting differences among cities and shifting of neonatal deaths into the latter period.

These data have not been available publicly for analysis. The analysis should provide new baseline information on the true outcome of pregnancies in biologically similar populations.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00833-02 EB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Outcomes of Deliveries with IUD Use During Conception

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary D. Overpeck Health Statistician EB/EBRP/NICHD

Other: George G. Rhoads Branch Chief EB/EBRP/NICHD

COOPERATING UNITS (if any)

Biometry Branch, EBRP, NICHD (H.J.Hoffman); CEB, CRP, NICHD (B.Stadel);  
University of North Carolina, Dept. of Epidemiology (E.Heineman).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

1

1

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

This study compares the outcome of deliveries (live births, fetal deaths, early neonatal deaths, and birth weight) of women who conceived with an IUD in place to deliveries without an IUD present. Data are available for deliveries occurring in 1975-1977 from the Kaiser-Permanente Birth Defects Study. The data base contains extensive information on maternal characteristics, patterns of contraceptive use and fetal outcome.













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Bethesda, MD 20892-1150  
301-496-1080

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